Testing for Co-Adaptation of Plants and Soil Organisms in a Biodiversity Experiment

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Summary

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Grasslands are widely used model ecosystems in biodiversity–ecosystem functioning research. A number of studies in the past decades have shown that increasing plant species diversity improves ecosystem functioning and thereby plant productivity in grasslands. Ecological mechanisms underlying these positive biodiversity effects are extensively studied but the potential role of natural selection and evolutionary mechanisms have rarely been considered. In Chapter 1, we studied whether long-term natural selection of plants increases biodiversity effects on productivity. To test this, we measured the productivity of plant communities selected over eight years in a grassland biodiversity experiment and compared it with the productivity of plant communities with identical species composition but without such common selection history. We found that community evolution increased plant community productivity at low but not at high diversity.

Associations between plants and soil microbes have received much attention in the past decades. However, very few studies have considered whether interactions between plants and such microbes may alter over ecological time-scales through adaptation. In Chapter 2, I hypothesized that such co-adaptation occurred in a long-term biodiversity experiment between plants and arbuscular mycorrhizal fungi (AMF). I tested the hypothesis using plants and AMF co-selected in plant monocultures or mixtures over 11 years. I show that co-adaptation between plants and AMF did occur in a long-term biodiversity experiment. In addition, I show that the outcomes of co-adaptation can be detrimental rather than beneficial to the performance of plants.

I continued studying associations between plants and soil microbes in Chapter 3 and tested whether selection of plants and soil microbes in plant monocultures vs. mixtures over 11 years leads to differences in plant phenotypes and microbiomes. In addition I tested whether co-adaptation occurred during this time between plants and soil microbiota excluding AMF. Selection in plant monocultures vs. mixtures led to differences in plant phenotypes and soil microbial compositions. I found that co-adaptation of plants and soil microbiota in monocultures, but not in mixtures, led to negative effects on plant performance. Finally I demonstrate that high soil biodiversity protects plants from these negative effects.

The results of this dissertation offer insights into the mechanisms underlying the positive relationship between biodiversity and ecosystem functioning and demonstrate that interactions between organisms can change over ecological time-scales. Overall, these results emphasize the importance of high below- and aboveground biodiversity for the optimal functioning of ecosystems.

Zusammenfassung

Wiesenlandschaften werden in Untersuchungen des Zusammenhangs zwischen Biodiversität und Ökosystemfunktionen oft als Modellökosystem genutzt. Verschiedene Studien haben während der letzten zwei Jahrzehnte gezeigt, dass eine höhere Pflanzendiversität die Funktion eines Ökosystems und somit die Produktivität der Pflanzen verbessern kann. Die ökologischen Mechanismen, die dieser Beobachtung zu Grunde liegen, wurden schon sehr genau untersucht. Allerdings wurde bisher die Untersuchung der Rolle von natürlicher Selektion und evolutionären Mechanismen vernachlässigt. Im ersten Kapitel haben wir untersucht, ob mehrjährige natürliche Selektion bei Pflanzen den Biodiversitätseffekt auf die Pflanzenproduktivität erhöht. Um diese Hypothese zu testen, haben wir die Produktivität von zwei Typen von Pflanzengemeinschaften verglichen. Die eine Pflanzengemeinschaft war in einem Wiesenbiodiversitätsexperiment über acht Jahre selektioniert worden, während die andere, die aus identischen Arten bestand, keine solche gemeinsame Vergangenheit der Arten hatte. Wir konnten zeigen, dass diese gemeinschaftliche Evolution die Produktivität bei geringer bis mittlerer Diversität erhöhte. Pflanzengemeinschaften mit der höchsten getesteten Diversität von 8 Arten zeigten keine entsprechenden Unterschiede, vielleicht weil die Evolution in diesen komplexeren Systemen mehr Zeit für adaptive Veränderungen gebraucht hätte.

Assoziationen zwischen Pflanzen und Bodenorganismen wurden in den letzten Jahrzehnten oft erforscht. Die zahlreichen Studien haben aber oft nicht beachtet, dass die Interaktionen zwischen Pflanzen und Mikroben sich relativ schnell während ökologisch relevanten Zeitspannen durch Adaptation verändern können. Im zweiten Kapitel habe ich getestet, ob in einem langfristigen Biodiversitätsexperiment eine Koadaptation zwischen Pflanzen und arbuskulären Mykorrhizapilzen stattgefunden hatte. Ich habe diese Hypothese mit Pflanzen und Mykorrhizapilzen getestet, welche zusammen entweder in Pflanzenmischungen oder Monokulturen für 11 Jahre selektioniert wurden. Ich konnte zeigen, dass tatsächlich eine Ko-adaptation zwischen Pflanzen und Pilzen erfolgte, diese jedoch für die Pflanzen nicht nutzbringend sondern im Gegenteil eher nachteilig war.

Im dritten Kapitel habe ich die Assoziationen zwischen Pflanzen und Bodenmikroben noch weiter untersucht. Ich testete, ob die Selektion von Pflanzen und Bodenmikroben in Pflanzenmonokulturen oder Mischungen über den Zeitraum von 11 Jahren zu Unterschieden im Pflanzenphänotyp oder im Pflanzen-Mikrobiom führte. Zusätzlich habe ich getestet, ob in dieser Zeit zwischen den Pflanzen und den Bodenorganismen (ohne arbuskuläre Mykorrhizapilze) eine Ko-Adaptation stattgefunden hatte. Ich konnte zeigen, dass die Selektion in Monokultur oder Mischung zu unterschiedlichen Pflanzenphänotypen und unterschiedlicher Zusammensetzung der Bodenorganismen führte. Ausserdem zeigte sich, dass diese Ko-Adaptation in Monokulturen negative Effekte auf die Pflanzenproduktivität hatte. Eine Ko-Adaptation zwischen Pflanzen und Bodenorganismen in Mischungen führte nicht zu diesem negativen Ergebnis. Letztlich zeigte ich, dass eine hohe mikrobielle Bodendiversität die Pflanzen vor solchen negativen Effekten beschützt.

Die Resultate dieser Dissertation bieten Einblicke in die Mechanismen, welche der positiven Beziehung zwischen Biodiversität und Ökosystemfunktionen zugrunde liegen. Sie zeigen, dass die Interaktionen zwischen Organismen sich während ökologisch relevanten

Zeitspannen verändern können. Meine Resultate verdeutlichen die Wichtigkeit der Erhaltung von ober- und unterirdischer Biodiversität für das optimale Funktionieren von Ökosystemen.

Introduction

General introduction

The world's biodiversity is rapidly declining, and the consequences on ecosystem functioning and ecosystem services are feared to be severe (Barnosky *et al.*, 2011; Cardinale *et al.*, 2012; Pimm *et al.*, 2014; Ceballos *et al.*, 2015). The major causes of the declining biodiversity include overexploitation of natural resources, fragmentation and loss of habitats, pollution, climate change and the consequent species invasions to non-native ecosystems (Barnosky *et al.*, 2011; Pimm *et al.*, 2014; Ceballos *et al.*, 2015; Isbell *et al.*, 2017). Loss of biodiversity can reduce a number of vital ecosystem functions, including plant productivity, decomposition, nutrient acquisition and recycling — and the stability of these functions (Cardinale *et al.*, 2012). Plants as primary producers and connectors of below- and aboveground biodiversities have an essential role in ecosystem functioning and services (Tilman & Downing, 1994; Naeem *et al.*, 1994; Daily, 1997; van Dam & Heil, 2011) but increasing evidence suggests an equally important role of soil microbiota (Schnitzer *et al.*, 2011; Maron *et al.*, 2011; Wall *et al.*, 2015; Bever *et al.*, 2015; Delgado-Baquerizo *et al.*, 2016). Although interactions between plants and soil microbes are intensively studied (van der Putten *et al.*, 2001; van der Heijden *et al.*, 2008; van Dam & Heil, 2011; van der Putten *et al.*, 2013; Delgado-Baquerizo *et al.*, 2016), long-term influence of biodiversity loss on these interactions and consequences on ecosystem functioning are not well understood, particularly because the interactions may alter over time.

A brief history of the biodiversity–ecosystem functioning research

The impact of biodiversity loss on ecosystem functioning has been an active topic of research for over two decades (Tilman & Downing, 1994; Tilman *et al.*, 1997; Huston, 1997; Balvanera *et al.*, 2006; Reich *et al.*, 2012; Zuppinger-Dingley *et al.*, 2014), but the original discovery of the positive correlation between biodiversity and ecosystem functioning is not as recent. Charles Darwin (1859) wrote "It has been experimentally proved, that if a plot of ground be sown with one species of grass, and a similar plot be sown with several distinct genera of grasses, a greater number of plants and a greater weight of dry herbage can thus be raised." It remains unclear whose finding Darwin refers to but the phenomenon he raises up in the book is currently a topic of strong interest in ecology. During the past decades a number of studies have re-confirmed the finding that productivity of a plant community increases with increasing plant species diversity (e.g. Tilman *et al.*, 1997; Balvanera *et al.*, 2006; Cardinale *et al.*, 2007). The positive relationship between plant biodiversity and productivity, becomes apparent when dry aboveground biomasses of plant communities in an area of a particular size are plotted against the number of plant species contributing to the community biomass. Ecologists call the phenomenon a positive biodiversity effect, or overyielding, a situation where plants grown in mixtures of species produce more biomass than the contributing species would in average produce in single-species communities, i.e. monocultures (e.g. Hooper & Dukes, 2003). If the species composition of these above-mentioned communities would be maintained and the aboveground biomass measured at regular intervals over multiple years, another fundamental phenomenon in plant community ecology might also become apparent: the slope steepens over time as the positive biodiversity effect strengthens (Cardinale *et al.*, 2007; Marquard *et al.*, 2009; Reich *et al.*, 2012).

Mechanisms underlying the positive biodiversity effects

The positive and strengthening biodiversity effects have been explained by several mechanisms. In principle, the productivity of a community often increases with the increasing number of occupied ecological niches. When species occupy different niches, resource competition between species is reduced and more resources can be extracted from the biotope for plant biomass production (Cardinale *et al.*, 2011). Co-existing species may achieve complementarity for instance by expressing differential height, specific leaf area or rooting depth and thereby avoid competition from light and nutrients, respectively (Roscher *et al.*, 2008; von Felten & Schmid, 2008; Zuppinger-Dingley *et al.*, 2014). In addition to the complementary resource use, positive biodiversity effects have been explained by stronger accumulation of resources at high diversity provided by the higher amount of decomposable material (Fornara & Tilman, 2008). Also, soil microbes modify the positive biodiversity effects: accumulation of specialized plant enemies in monocultures, and their dilution in diverse plant communities, reduces plant productivity at low in comparison with high plant species richness (Mordecai, 2011; Kulmatiski *et al.*, 2012; Eisenhauer *et al.*, 2012). In addition, positive biodiversity effects have been explained by a sampling effect, suggesting that the probability that particularly productive species exist in the community increases with increasing species richness (Tilman *et al.*, 1997; Huston, 1997).

Biodiversity experiments

Grasslands are widely used model ecosystems in the research of biodiversity and ecosystem functioning but, in addition, studies have been conducted in forests (Paquette & Messier, 2011; Liang *et al.*, 2016), drylands (Maestre *et al.*, 2012) and marine ecosystems (Duffy *et al.*, 2016). The research in biodiversity and ecosystem functioning originates from studies observing natural communities (reviewed by Eisenhauer *et al.*, 2016). One of the early experimental biodiversity studies manipulated naturally occurring species using nitrogen application (Tilman & Downing, 1994). The potential influence of fertilization on productivity was, however, criticized (Givnish, 1994). This led to an establishment of biodiversity experiments where plant communities were sown with various species compositions and diversities (reviewed by Cardinale *et al.*, 2012). Examples of such experiments are the Cedar Creek experiment in Minnesota, U.S. (Tilman *et al.*, 1996), the ECOTRON experiment in England (Naeem *et al.*, 1994), the BIODEPTH experiment conducted across Europe (Hector, 1999) and the Jena Experiment in Jena, Germany (Roscher *et al.*, 2004).

Soil biodiversity and ecosystem functioning

Biodiversity in the soil is linked to multiple essential ecosystem functions. For instance, soil microbes carry out decomposition, provide nutrients to plants, increase productivity, protect plants from multiple environmental stressors and help plants defending against enemies (Lugtenberg & Kamilova, 2009; Compant *et al.*, 2010; Smith & Smith, 2011; Ahemad & Kibret, 2014; Wagg *et al.*, 2014). Studies have demonstrated that climate change, intensive agriculture and pollution, for example, may harm soil biodiversity remarkably (Gosling *et al.*, 2006; Tsiafouli *et al.*, 2015; Wall *et al.*, 2015). Loss of soil biodiversity can reduce the optimal functioning of ecosystems by breaking up networks of organisms and by disturbing

processes carried out by soil microbes (Gosling *et al.*, 2006; Wagg *et al.*, 2014). In addition, loss of soil biodiversity may expose plant communities to soil-borne diseases from which they are normally protected by the large biodiversity in the soil (Wardle & Yeates, 1993; Whipps, 2001; Oerke & Dehne, 2004; Eisenhauer *et al.*, 2012; van der Putten *et al.*, 2013; Ahemad & Kibret, 2014).

Interactions between plants and soil microbes

Plants interact with a remarkable amount of soil microbes in the rhizosphere, a narrow zone of soil surrounding plant roots (Whipps, 2001). Microbial composition in the rhizosphere is influenced by local biotic and abiotic conditions, which plants may alter by a secretion of chemical compounds, inputs of organic matter, root morphology, changes in soil moisture, temperature and pH and by providing resources and habitats to soil organisms (Bardgett & Wardle, 2003; Berg & Smalla, 2009; van Dam, 2009; van der Putten *et al.*, 2013; Bulgarelli *et al.*, 2013; Latz *et al.*, 2016). While plants may alter microbial composition in the soil, the composition of soil microbes can, in turn, drive changes in the plant community composition by differentially influencing the growth and survival of individual plants (van der Putten *et al.*, 2013). The responses of plants to soil microbes may vary from positive to negative (van der Putten *et al.*, 2013) and these responses are here called as positive and negative plant–soil feedbacks, respectively.

Drivers of positive plant–soil feedbacks

The soil microbes that typically contribute towards the positive plant–soil feedbacks are arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) (including nitrogen-fixing bacteria) (Smith & Smith, 2011; Ahemad & Kibret, 2014). AMF are soil-born fungi from the division of Glomeromycota that form symbiotic interactions with 80 % of the land plants (Smith & Smith 2011). In this symbiosis, AMF receives carbon from the host plant and in exchange provides several benefits to the host, including increased uptake of mineral nutrients (Gianinazzi-Pearson, 1996; van der Heijden *et al.*, 2006; Smith & Smith, 2011), cover from abiotic stressors such as drought (Augé, 2001) and protection from the detrimental effects of below- and aboveground pests (Newsham *et al.*, 1995; Azcón-Aguilar & Barea, 1997; Rodriguez & Redman, 2008; Vannette *et al.*, 2013). Although AMF are able to promote plant growth, in reality the outcome of the symbiosis can vary from mutualism to parasitism (Johnson *et al.*, 1997; Klironomos, 2003; Kiers & van Der Heijden, 2006; Argüello *et al.*, 2016), mainly depending on the amount of carbon that the fungus extracts (Pringle, 2016). PGPR, in turn, comprise 2–5 % of bacteria existing in the rhizosphere (Ahemad & Kibret, 2014). Directly, PGPR may improve plant growth by altering plant hormone levels, promoting a secretion of different root-produced substances to the rhizosphere and mobilizing essential nutrients of plants (Hayat *et al.*, 2010; Ahemad & Kibret, 2014). PGPR can promote plant growth also indirectly by producing antifungal substances against soil-borne fungal enemies of plants, inducing plant defense responses and competing against pathogenic microbes in the available niches in the rhizosphere (Lugtenberg & Kamilova, 2009; Glick, 2012).

Drivers of negative plant–soil feedbacks

Negative plant–soil feedbacks, in turn, are often caused by soil pathogens (van der Putten *et al.*, 2013; Bever *et al.*, 2015). Soil pathogens represent a wide group organisms including bacteria, fungi, viruses and water-molds, which can cause negative effects on plant growth and survival for instance through seed decays, mortality of seedlings or root necrosis (Bever *et al.*, 2015). Pathogens are often classified as generalists (many host species) and specialists (one or few host species) depending on the range of hosts that they consume (Mordecai, 2011). Increasing evidence suggests that, among the various soil organisms that plants interact with, specialized pathogens play a particularly important role in the structuring of plant communities (Petermann *et al.*, 2008; Mordecai, 2011; Maron *et al.*, 2011; Bever *et al.*, 2015). The reason is a Janzen-Connell effect, a tendency of specialized pathogens to accumulate near-by a dominant species of a community (Mordecai, 2011). The phenomenon was named after the two authors who first discovered that specialized aboveground pathogens and seed predators limit the growth of tree seedlings if grown nearby the conspecific adult trees (Janzen, 1970; Connell, 1971; van der Putten *et al.*, 2013). Later, the Janzen-Connell effect was found to occur also among belowground pathogens (Petermann *et al.*, 2008; Mangan *et al.*, 2010). Due to Janzen-Connell effects, the consequences of pathogen accumulation in monocultures are particularly strong, whereas in diverse plant communities the negative effects of specialized pathogens are often diluted (van der Putten *et al.*, 2013).

Plant resource allocation

In addition to being protected from enemies by beneficial organisms, plants themselves act against such organisms by producing defensive secondary metabolites or structures. (e.g. van der Meijden *et al.*, 1988; Herms & Mattson, 1992; van Dam & Heil, 2011). Considering that plants have limited amount of resources in use, investment of resources in defenses should reduce the amount of resources available for growth and *vice versa*. Such a growth–defense tradeoff is thought to define resource allocation pattern in plants (Coley *et al.*, 1985; Herms & Mattson, 1992). A recent study found after eight years of selection in plant monocultures or mixtures of a biodiversity experiment that plant–soil feedbacks of monoculture soil were positive to plants selected in monocultures but negative to plants selected in mixtures (Zuppinger-Dingley *et al.*, 2016). The negative response of plants selected in mixtures suggested that pathogens had accumulated in the tested monoculture soil over time, as predicted by the Janzen-Connell effect. The positive response of plants selected in monocultures, in turn, suggested that these plants were less vulnerable to the pathogens. The authors hypothesized that selection in monocultures might have favoured plants that trade off growth for increased investment in defenses against the pathogens or, alternatively, plants that are better protected from the pathogens by beneficial soil organisms.

The influence of natural selection on positive biodiversity effects

Selection in species mixtures of a biodiversity experiment, instead, was recently found to increase niche complementarity between co-selected plant species (Zuppinger-Dingley *et al.*, 2014). In this study, plants selected in species mixtures, in comparison to monocultures, showed higher variation in height and specific leaf area between species. Moreover, plants selected in species mixtures showed increased biodiversity effects, which the authors

proposed to have taken place through increased niche differentiation and reduced competition between plant species. Presumably, the formation of such diversity-adapted plant types had occurred through an environmental filtering from an initially larger standing variation of individuals: individuals whose traits fitted relatively better to the local biotic and abiotic conditions had higher changes to survive and thereby increase their genetic representation in the community (Bossdorf et al., 2008). Because the process changes allele frequencies among species co-existing in the community (Fakheran et al., 2010) similarly to genetic recombination or mutations (Anderson et al., 2011), such changes in the community can be referred to as community evolution (Whitham et al., 2006). So far, evidence for community evolution has only been found in the communities of plankton and bacteria (Yoshida et al., 2003; Lawrence et al., 2012; Fiegna et al., 2014, 2015). The finding by Zuppinger-Dingley *et al*. (2014), however, introduced an evolutionary aspect to the discussion of ecological processes underlying positive biodiversity effects in plant communities (Tilman & Snell-Rood, 2014) and clarified the necessity to consider the positive and strengthening biodiversity effects also in the light of community evolution.

Thesis outline

The present dissertation focuses on studying the impacts of natural selection on plant and soil communities in response to local plant species diversity. In Chapter 1 we ask whether natural selection increases the productivity of plant communities. To test this we re-assemble plant communities co-selected in a grassland biodiversity experiment over eight years and compare the productivity with newly assembled plant communities of identical species composition but without such co-selection history. To additionally examine the potential influence of soil microbiota, we test the productivity of the plant communities in the presence and absence of co-selected soil microbes.

Plants selected in monocultures in a biodiversity experiment had evolved positive plant–soil feedbacks after eight years, which could have been due to co-adaptation with AMF and increased pathogen defense (Zuppinger-Dingley *et al.* 2016). In Chapter 2 I hypothesize that long-term co-selection in a biodiversity experiment can lead to a co-adaptation between plants and AMF or, alternatively, selects plants for increased defense. To test my hypothesis I set up a plant–soil feedback experiment using plants selected over 11 years in plant monocultures or mixtures and correspondingly co-selected plant-monoculture and mixture AMF from the same plots of the biodiversity experiment. To assess the responses of plants and AMF I measured multiple plant traits and estimated AMF colonization rate in the plant roots.

In Chapter 3, I hypothesize that co-selection of plants and soil microbes over 11 years in plant monocultures and mixtures of a biodiversity experiment leads to differences in plant functional traits and microbial composition. In addition, I hypothesize that soil microbes from plant monocultures or mixtures alter plant performance differentially in “home” and “away” combinations with plants selected in the same monocultures or mixtures. To test these hypotheses I set up another plant–soil feedback experiment using plants selected over 11 years in monocultures or mixtures in a biodiversity experiment. I grew the plants in sterilized soil inoculated with full, simplified or sterilized soil microbes from corresponding monoculture or mixture plots of the biodiversity experiment. I assessed the feedbacks of

plants and soil organisms by conducting plant trait measurements, sequencing bacterial soil microbiota and by estimating the rate of AMF colonization in the plant roots.

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Chapter 1:
Community evolution increases plant productivity at low diversity

Community evolution increases plant productivity at low diversity

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Bernhard Schmid, Dan F.B. Flynn and Gerlinde B. De Deyn conceived the project;
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ABSTRACT

Species extinctions from local communities can negatively affect ecosystem functioning. Ecological mechanisms underlying these impacts are well studied but the role of evolutionary processes is rarely assessed. Using a long-term field experiment, we tested whether natural selection in plant communities increased the effects of biodiversity on productivity. We re-assembled communities with 8-year co-selection history adjacent to naïve communities with identical species composition but no history of co-selection. Mixtures of two to four co-selected species were more productive than their corresponding naïve communities over four years in soils with or without co-selected microbial communities. At the highest diversity level of eight plant species, no such differences were observed. Our findings suggest that plant community evolution can lead to rapid increases in ecosystem functioning at low diversity but may take longer at high diversity. This effect was not modified by treatments that simulated additional co-evolutionary processes between plants and soil organisms.

Key words: biodiversity, community evolution, co-selection, ecosystem functioning, grassland species, Jena Experiment, plant productivity, soil organisms

INTRODUCTION

A large number of experiments have shown that species richness positively influences ecosystem functioning, in particular plant biomass production (Tilman *et al.*, 1997; Balvanera *et al.*, 2006; Cardinale *et al.*, 2007, 2012; Reich *et al.*, 2012; Meyer *et al.*, 2016). These biodiversity effects have been explained by sampling effects that increase the chance of including productive species in diverse communities (Tilman *et al.*, 1997; Huston, 1997) or by complementary effects between species, which allow mixtures to extract resources from the environment more efficiently (Roscher *et al.*, 2008; Mueller *et al.*, 2013). Furthermore, diversity-dependent reductions in soil fertility (Fornara & Tilman, 2008) or density-dependent accumulations of specialist pathogens over time (Schnitzer *et al.*, 2011) have been shown to contribute to decreasing productivity at low plant diversity and in plant monocultures.

Complementarity effects between co-occurring species increase over time (Cardinale *et al.*, 2007; Fargione *et al.*, 2007; Reich *et al.*, 2012; Meyer *et al.*, 2016). Evidence that this might be due to evolutionary processes in plant communities has been found in a glasshouse experiment comparing the performance of populations selected in monocultures vs. diverse plant communities in newly assembled test monocultures and two-species mixtures (Zuppinger-Dingley *et al.*, 2014). This suggests that community evolution may shape diversity–productivity relationship more generally, which could be tested if entire communities of co-selected plant species would be compared with communities of the same plant species but without co-selection history. Community evolution has been defined as genetically based changes among species constituting the community, which alter species performances and interactions (Whitham *et al.*, 2006). Such changes may occur via genetic recombination, mutations (Anderson *et al.*, 2011), or a sorting-out from standing genetic variation through differential survival and growth of individuals (Fakheran *et al.*, 2010). Natural selection can lead not only to changes in gene frequencies in populations within species, but selection at the level of communities can in addition lead to correlated changes in gene frequencies in multiple species (Whitham *et al.*, 2006) in response to one another or to co-varying environmental conditions. But empirical evidence for community evolution so far has only been demonstrated in planktonic and bacterial communities (Yoshida *et al.*, 2003; Lawrence *et al.*, 2012; Fiegna *et al.*, 2014, 2015) and not yet in higher plants. Here we report results from a field experiment where we tested whether plant community evolution influences plant community productivity.

Recent evidence suggests selection of particular genotypes from the total genetic pool of a species may affect ecosystem functioning in field experiments (Strauss *et al.*, 2008; Lipowsky *et al.*, 2011; Lau & Lennon, 2012; Kleynhans *et al.*, 2016; Rottstock *et al.*, 2017). We propose that selection of genotypes from the gene pool of entire communities may affect ecosystem functioning if non-random niche or trait changes in response to other phenotypes in the community result in reduced niche overlap and a more complete use of biotope space (Dimitrakopoulos & Schmid, 2004; Jousset *et al.*, 2011), thus leading to increased plant community productivity. We therefore compared the productivity of plant communities assembled from plants which have co-occurred for eight years in a long-term grassland biodiversity experiment (the Jena Experiment(Roscher *et al.*, 2004) with the productivity of

plant communities of identical species composition, but without any co-occurrence history ("naïve communities"). The naïve plants were obtained from the seed supplier of the original seeds used to establish the Jena Experiment. We used experimental plant monocultures and 2-, 4- or 8-species mixtures with twelve different species compositions for each diversity level.

Plant community evolution in the field may also depend on the local environment, such as the soils in which co-evolution with soil microorganisms occurred. For instance, plant–soil feedback experiments have shown that soil biota change in response to different plant species, which can in turn modify the composition and productivity of plant communities (Klironomos, 2002; Kardol *et al.*, 2007; Wagg *et al.*, 2015). To assess whether additional co-evolutionary processes between plants and soil organisms modified plant community evolution, we grew the selected and naïve plant communities in soils with co-selected soil organisms (native soil) and with external soil organisms (neutral soil; see Methods and Fig. S1). Community-level plant productivity was measured each year from 2012 to 2015 by collecting species-specific aboveground biomass at the time of peak biomass in spring (see Methods).

METHODS

Study site

The present study was conducted at the Jena Experiment field site (Jena, Thuringia, Germany, 51˚N, 11˚E, 135m a.s.l.) from 2011 to 2015. The Jena Experiment is a long-term biodiversity field experiment located in the floodplain of the river Saale where 60 Central European grassland species have been grown in a number of species combinations since 2002 (Roscher et al. 2004).

Community-evolution treatment

The 48 experimental plant communities of this study included twelve monocultures (of which one had to be removed from all analyses because it was planted with the wrong species), twelve 2-species mixtures, twelve 4-species mixtures and twelve 8-species mixtures. We used two community-evolution treatments; plants with eight years of co-selection history in 48 different plant communities in the Jena Experiment (communities of co-selected plants) and plants without such co-selection history in the Jena Experiment (naïve communities). The plant seeds of naïve communities were obtained from the same commercial seed supplier (Rieger Hofmann GmbH, in Blaufelden-Raboldshausen, Germany) as the seeds used for the establishment of the original communities of the Jena Experiment. This supplier collected plants of the different species at field sites in Germany and propagated them for at least five years in monoculture, reseeding them every year. Seeds of communities of co-selected plants were produced in an experimental garden in Zurich, Switzerland, from cuttings that had been made in the Jena Experiment and were then planted in Zurich in the original species combination in plots fenced with plastic netting to reduce pollination between communities. To obtain sufficient numbers of seeds from communities of co-selected plants, a small number was additionally collected directly in the plots of the Jena Experiment. All these

seeds were thus offspring of plant populations that had been sown in 2002 and grown until 2010 in plots of the Jena Experiment.

The seeds of communities of co-selected plants and naïve communities were germinated in potting soil (BF4, De Baat; Holland) in mid-January 2011 in a glasshouse in Zurich. In March 2011, the seedlings were transported back to the field site of the Jena Experiment and planted within 2 x 2 m subplots of the original plots (Fig. S1). There were four 1 x 1 m quadrats with different soil treatments in each (see next section). Each quadrat was further divided into two 1 x 0.5 m halves. The seedlings of communities of co-selected plants were transplanted into one half and seedlings of naïve communities into the other half of each quadrat at a density of 210 plants per m^2 with a 6-cm distance between individuals in a hexagonal pattern (Fig. S1). Species were planted in equal proportions, but if a species was no longer present in an original plot of the Jena Experiment it was excluded from both communities of co-selected plants and naïve communities. Five plant species were excluded in total. The seedlings received water every second day for six weeks after transplanting to ensure the plants established.

Soil treatment

Within each 2 x 2 m subplot of the 48 plots of the Jena Experiment used for the present study, the original plant cover was removed in September 2010 (and used for the plant propagation in the experimental garden in Zurich, see previous section), and the soil was excavated to a depth of 0.35 m and sieved. To minimize exchange of soil components between quadrats within subplots and with the surrounding soil, two 5-cm layers of sand were added to the bottom of the plots and separated with a 0.5 mm mesh net. The borders of the quadrats and the subplots were separated by plastic frames (Fig. S1). Using the excavated original soil from each of the plots, four soil treatments were prepared. First, half of the soil (approximately 600 kg per plot) was gamma-sterilized to remove the original soil community. Half of the gamma-sterilized soil was then inoculated with 4 % (by weight) of live sugar-beet soil and 4 % of sterilized original soil of the corresponding plot (“neutral soil” obtained by inoculation). Live sugar-beet soil was added to create a natural, but neutral soil community and was previously collected in an agricultural sugar-beet field not associated with the Jena Experiment, but with comparable soil properties. The other half of the gamma-sterilized soil was inoculated with 4 % (by weight) of live sugar-beet soil and 4 % of live original soil of the corresponding plot (“native soil” obtained by inoculation). The other half of the soil was unsterilized and used for the other two soil treatments. Half of this soil was filled back into one quadrat of the corresponding plot (“native soil”). The other half of the unsterilized soil was mixed among all plots and filled into the remaining quadrats. This fourth soil treatment was abandoned after two years because the plant community was excavated for another experiment. Therefore, this treatment is not included in the present study.

Before the soils were added into the quadrats in December 2010, they were rested in the field in closed bags to allow for the soil chemistry to equalize and to encourage soil biota of the inocula to colonize the sterilized soil before planting. After the soil was added, all quadrats were covered with a net and a water permeable black sheet to avoid spilling between quadrats until the seedlings were transplanted in March 2011.

Data collection

We maintained the test communities by weeding three times a year and by cutting the plants twice a year at typical grassland harvest times (late May and August) in central Europe. To measure productivity, we harvested plant material 3 cm aboveground from a 50 x 20 cm area in the centre of each half-quadrat, sorted it into species, dried it at 70°C and weighed the dry biomass.

SLA measurements

At the end of the experiment, in May 2015, we measured specific leaf area (SLA) for 30 species in neutral soil. For each species, we collected up to 20 representative leaves (depending on the leaf size of the species) from four individuals and measured the leaf area by scanning fresh leaves with a Li-3100 Area Meter (Li-cor Inc., Lincoln, Nebraska, USA) immediately after harvest and determining the mass of the same leaves after drying.

T-RFLP assay

Terminal restricted fragment length polymorphism (T-RFLP) targeting the 16S RNA was used to characterize the composition of the soil bacterial communities (Liu *et al.*, 1997). In April 2011, four soil samples per quadrat were extracted and pooled to assess the establishment of soil microbial communities and to test whether soil treatments were distinct. In 2012, a further set of soil samples was taken and analysed to confirm the establishment of different soil biotic treatments. T-RFLP soil analyses revealed that bacterial communities of the soil treatments remained distinct: each soil treatment had a characteristic bacterial composition both one and two years after planting, with some overlap (Table S3).

Statistical analysis

We analysed the data from the four spring harvests 2012, 2013, 2014 and 2015, which corresponded to peak aboveground plant biomass values. We analysed plant biomass (g/m^2) as a function of the design variables using mixed models and summarized results in analyses of variance (ANOVA) tables (e.g. Table S1). Significance tests were based on approximate F-tests using appropriate error terms and denominator degrees of freedom.

The fixed terms in the model were species richness of the original plots of the Jena Experiment (factor with 4 levels: facSR), year of harvest (factor with 4 levels: Har), soil treatment (factor with 3 levels: SH), community-evolution treatment (communities of co-selected plants vs. naïve communities: PH) and interactions of these. The random terms were plot, quadrat, half-quadrat and their interactions with year of harvest. Statistical analyses were conducted using the software R, version 3.2.3 (R Core Team 2015). Mixed models using residual maximum likelihood (REML) were fitted using the package ASReml for R (Butler 2009).

Within-species variation in SLA was calculated as the within-species variance component for each community (residual mean square after fitting species). We had insufficient trait data to test for increased between-species variation in communities of co-selected plants containing a mixture of species.

The calculation of operational taxonomic units (OTUs) from the T-RFLP raw data (restriction enzyme products) was done using the T-RFLP processing software T-REX

(Culman *et al.* 2009) for each soil treatment and year separately and the soil-specific outputs were then compared with an analysis of similarities (anosim() function of the vegan package (Oksanen *et al.* 2016).

RESULTS

Overall, for each doubling of species richness community aboveground biomass increased by 100 $g \cdot m^{-2} \cdot y^{-1}$, a typical value for grassland biodiversity experiments (Hector *et al.*, 1999). In general, communities of co-selected plants were more productive than naïve communities of the same species composition. The significant interaction between species richness and community-evolution treatment or in short plant history ($F_{3,191.2}$ = 2.77, P = 0.043; Table S1a) indicated that this was mainly due to increased productivity of 2- and 4-species mixtures and a smaller increase in monocultures of co-selected plants. In contrast, 8-species mixtures of co-selected or naïve plants were equally productive (Fig. 1a). The calculated relative productivity (percentage of the mean productivity of 8-species mixtures for each plant history-by-soil treatment-by-year combination) confirmed that especially 2- and 4-species mixtures of co-selected plants increased productivity relative to 8-species mixtures ($F_{3,191.9}$ = 2.90, P = 0.036; Fig. 1b; Table S1b). The positive effect of community evolution on relative productivity was significantly larger in 2- and 4-species mixtures than in monocultures ($F_{1,43.7}$ = 6.37, P = 0.015 for the interaction between plant history and the contrast of "2- or 4-species mixtures vs. others"). The differences in relative productivity between communities of co-selected plants and naïve communities increased over time for these low-diversity mixtures as well as for monocultures in all three soils (Fig. 2). For monocultures, this was due to the deteriorating performance of naïve plants, possibly due to the accumulation of soil pathogens, whereas for 2- and 4-species mixtures it was due to an increasing relative performance of communities of co-selected plants.

To test whether the communities of co-selected plants were particularly productive in 2- and 4-species mixtures at the beginning of the Jena Experiment (i.e. when they were "naïve" communities themselves), we compared the productivity data of 2003–2006 with the data of 2012–2015. To standardize for differences in overall productivity between time periods we again used relative productivity (percentage of mean of 8-species mixtures per year). The plant communities were established in neutral soil in 2002 at the beginning of the experiment. We therefore used only data from neutral soil from 2012 to 2015. The communities of co-selected plants were significantly different in their response compared to the two types of naïve communities because of their increased relative productivity in 2- and 4-species mixtures ($F_{1,46.5}$ = 5.73, P = 0.021 for the interaction of plant history with the contrast "2- or 4-species mixtures vs. others"; Fig. S2). Differences between the communities of the naïve ancestors of the co-selected plants and our current re-assembled naïve plant communities were small and not significant ($F_{1,46.1}$ = 0.23, P = 0.637 for the interaction of the contrast "naïve ancestors vs. current naïve communities" with the contrast "2- or 4- species mixtures vs. others").

Plant community productivity was initially greater in inoculated soils, in particular at high diversity, which was reflected in an overall main effect of soil treatment and significant

interactions with year, and with year and species richness (Table S1). This was probably caused by the nutrient flush associated with gamma-sterilization of the soil (Gebremikael *et al.*, 2015). But we found no evidence that our soil treatments modified the differences in biodiversity effects between communities of co-selected plants and naïve communities ($F_{1,183}$= 0.27, P = 0.847 and $F_{1,183.8}$= 1.401 P = 0.244 for the three-way interactions of plant history with species richness and the soil-treatment contrasts neutral vs. native and sterilized native vs. unsterilized native, respectively).

To explore potential mechanisms for the increased biodiversity effects in 2- and 4-species mixtures of co-selected plants, we calculated the proportional increase (decrease) in plant productivity for each community composition and soil treatment as the log ratio between communities of co-selected plants and naïve communities (Fig. 3). As expected, there was no increase in productivity in 8-species mixtures, but a strong increase in 2-species mixtures followed by 4-species mixtures (which had a higher absolute increase than 2-species mixtures, see Fig. 1a) and monocultures. Using contrasts between the different diversity levels, we could confirm that the three low diversity levels were significantly different form the 8-species mixtures ($F_{1,37.1}$ = 5.34 and P = 0.026). Among the three low diversity levels, the 2-species mixtures had significantly greater log ratios than 4-species mixtures and monocultures ($F_{1,39.2}$ = 4.44, P = 0.042).

Next, we tested whether the presence of particular plant functional groups influenced the increase in productivity in communities of co-selected plants at the 2- and 4-species richness levels; especially as legumes are known to drive over-yielding in grasslands (Spehn *et al.*, 2002). The presence of legumes and other plant functional groups, however, did not provide any further explanation for our results. Species-level productivity within communities was higher for the majority of plant species with a co-selection history, irrespective of functional-group identity (Fig. 4). Naïve communities showed more even species abundance distributions ($F_{1,132.2}$ = 4.28, P = 0.041; Table S2), mainly due to the lower evenness of communities of co-selected plants in the unsterilized native soil treatment (Fig. S3). Over the course of the experiment, evenness decreased similarly in communities of co-selected plants and naïve communities (Table S2).

Finally, we analysed changes in within-species trait variation along the species richness gradient as a potential mechanism contributing to the difference in productivity between communities of co-selected plants and naïve communities (Siefert *et al.*, 2015). Within-species variation in specific leaf area (SLA) decreased for communities of co-selected plants and increased for naïve communities with increasing species richness (Fig. 5; $F_{1,69.2}$ = 4.87, P = 0.031 for interaction of log species richness with plant history).

DISCUSSION

Our results show that eight years of community evolution in a biodiversity experiment can increase biodiversity effects on community productivity, suggesting that this may at least in part explain why biodiversity effects commonly increase over time in such experiments (Cardinale *et al.*, 2007; Fargione *et al.*, 2007; Reich *et al.*, 2012; Meyer *et al.*, 2016). The greater productivity in communities consisting of co-selected plants compared to

communities consisting of naïve plants was particularly evident in communities comprised of two or four species. One might claim that these effects were because we purchased the plant material of co-selected and naïve plants at two different points in time. We argue that this is not the case for the following reasons. First, co-selected and naïve plants were obtained for 52 different species and for each of them there were different community-specific co-selection histories. Second, 8-species mixtures with and without co-selection history showed the same productivity. In other words, because the positive effect of the community-evolution treatment was not statistically evident in the 8-species mixtures but strong in 2- and 4-species mixtures, this effect was unlikely simply due to initial differences in plant material.

Why was the community-evolution treatment not effective at the highest richness level tested? It is conceivable that selection pressure was dampened in communities where more than four species co-occurred. For instance, during initial establishment in a diverse community, each individual can have a very different set of immediate neighbours that could constrain the consistency in the selection pressure on individuals within a community. With fewer species in a mixture, the potential for the evolution of increased complementarity between plant species should be greater, given the relative constancy of the neighbours any given plant experiences. The greater proportional (but not absolute) increase of productivity in communities of co-selected plant species at the 2- than at the 4-species richness level, and the absence of such an increase at the 8-species richness level, are compatible with the idea that evolution for co-adaptation is stronger at low than at high diversity. At low diversity, intraspecific densities are higher and thus the chance for a uniform selection pressure across all intraspecific individuals is greater. As a consequence, there might be an upper limit of species richness beyond which selection is unlikely to strengthen biodiversity effects (Cardinale *et al.*, 2012). Additionally, community evolution leading to increased plant growth and productivity in diverse mixtures may be at the expense of reduced pathogen defense (Lemmermeyer *et al.*, 2015).

The performance of the naïve communities in the current study over the four years was comparable to the initial performance of the ancestral community of the co-selected plants (2003–2006). This similarity supports the view that the observed results at 2- and 4-species richness levels in communities of co-selected compared with communities of naïve plants are likely due to diversity-dependent community evolution. Indeed, the naïve communities did not catch up with the communities of co-selected plants during the course of the current experiment and differences in productivity from 2012 to 2015 even increased between the two community-evolution treatments (Fig. 2). With regard to underlying evolutionary mechanisms, this suggests that in our study community evolution was not or at least not solely due to an immediate sorting out of genotypes from standing variation (Fakheran *et al.*, 2010) during seedling establishment and initial growth.

The driving force behind community evolution for greater productivity at low diversity could have been related to particular species compositions (Zuppinger-Dingley *et al.*, 2014). There was, however, no evidence for any plant functional-group specific effect typically found in other contexts of biodiversity–ecosystem functioning research (Hooper & Vitousek, 1997; Spehn *et al.*, 2002). In fact, the majority of species produced greater biomass in communities of co-selected plants and evenness was only slightly reduced in these communities compared with communities of naïve plants.

Intraspecific variation in SLA decreased in communities of co-selected plants and increased in naïve communities with increasing species richness (Fig. 5), a result in line with previous findings for SLA in grassland species (Gubsch *et al.*, 2011). The increased within-species variation in monocultures suggests an evolutionary broadening of niches to benefit from a wider range of light conditions. In contrast, within-species trait variation may be less important in mixtures, due to the inherently lower intraspecific density at greater richness. The narrowing of within-species variation with increasing diversity in communities of co-selected plants could be an expected consequence of character displacement between species (Zuppinger-Dingley *et al.*, 2014). In relative terms, it seemed that species in naïve communities had not yet responded to different diversity treatments with an adjustment of within-species variation in the four years of this study. A more heterogeneous biotic environment may have caused their higher variation at high diversity.

Selected plants also had greater productivity than naïve plants in monoculture. The adaptation of selected plants to monoculture environments could have been due to the evolution of increased (belowground) pathogen defense (Zuppinger-Dingley *et al.*, 2016) or greater niche width (Bazzaz, 1996). Assuming soil-borne plant pathogens accumulated over time (Schnitzer *et al.*, 2011), in particular in the initially sterilized treatments, the decrease in monoculture productivity in naïve communities (Fig. 2) would be consistent with the hypothesis of increased pathogen defense in selected communities (Zuppinger-Dingley *et al.*, 2016). Assuming a correlation between resource-uptake and trait-based niches (Roscher *et al.*, 2015), the increase in within-species variation in SLA in monocultures of selected plants (Fig. 5) would be consistent with the second explanation related to niche width.

Positive plant diversity–productivity relationships may not only be driven by complementary resource use, and thus increased performance at high diversity (Roscher *et al.*, 2008; Mueller *et al.*, 2013), but also by pathogen accumulation in the soil and thus reduced performance at low diversity (Schnitzer *et al.*, 2011). Previous studies in the context of biodiversity–ecosystem functioning research have reported negative plant–soil feedbacks in native as opposed to neutral soils (Klironomos, 2002; Petermann *et al.*, 2008; Cortois *et al.*, 2016). Consequently, an increase of biodiversity effects during community evolution could also be due to the presence of co-selected soil biota. In our study, however, the outcome of the community-evolution treatment in mixtures was largely independent of the presence of co-selected soil biota. The generally lower productivity for both communities of co-selected plants and naïve communities in native soil, and with time in neutral soil, may have occurred through nutrient depletion or pathogen accumulation in all soil treatments. It is conceivable that co-evolution of plants with soil biota in our experimental systems was not effective because the large population sizes and short generation times of most soil organisms contributed to the re-assembly and fast evolution of soil communities (Lau & Lennon, 2012). Another explanation could be that microbes were dispersed via wind-blown particles to adjacent plots thereby potentially making the microbial communities less different in composition than if the plots would have been separated more in space.

Changes in the performance of individual species selected in different species diversity levels and tested under experimental abiotic or biotic conditions have been observed in previous studies (Lipowsky *et al.*, 2011; Zuppinger-Dingley *et al.*, 2014; Kleynhans *et al.*, 2016; Rottstock *et al.*, 2017). In our study, we demonstrated for the first time that changes in

the performance of entire plant communities over time depend on a history of co-selection among the plants species of the assembled mixtures. We suggest that these changes are the result of community evolution because they were maintained through seed production in an experimental garden and propagation of seedlings in a glasshouse to the replanting of communities in the field. However, we cannot exclude maternal carry-over and epigenetic changes (Verhoeven *et al.*, 2016) as additional potential evolutionary mechanisms. Independent of the mechanism, an ecosystem with individuals adapted to optimize the use of the local resources by reducing interspecific competition will be a well-functioning and sustainable system. Our new findings suggest that it is not sufficient to preserve species outside a community context for the conservation of biodiversity and its beneficial influence on ecosystem functioning and services. To protect species interactions and ecosystem functioning more efficiently, novel strategies should consider the conservation of entire communities or at least subsets of these. Our results emphasize that this is especially critical for less diverse communities, which may already suffer from the loss of some of their constituents.

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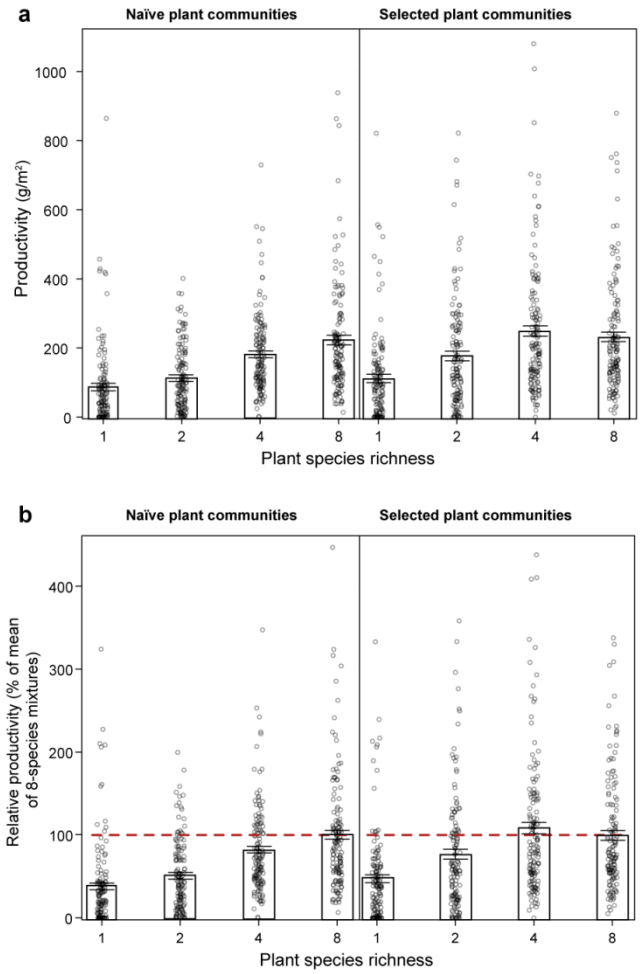

Figure 1 Community productivity for naïve communities and communities of co-selected plants at different species-richness levels. **(a)** Peak community aboveground biomass (g/m^2). Communities of co-selected plants (right panel) had slightly increased productivity in monocultures, more strongly increased productivity in 2- and 4-species mixtures, but similar productivity in 8-species mixtures as naïve communities (left panel). **(b)** as in (a) but showing relative productivity (% of mean productivity of 8-species mixtures per plant history-by-soil treatment-by-year combination). Means and standard errors are shown. Raw data plotted as points.

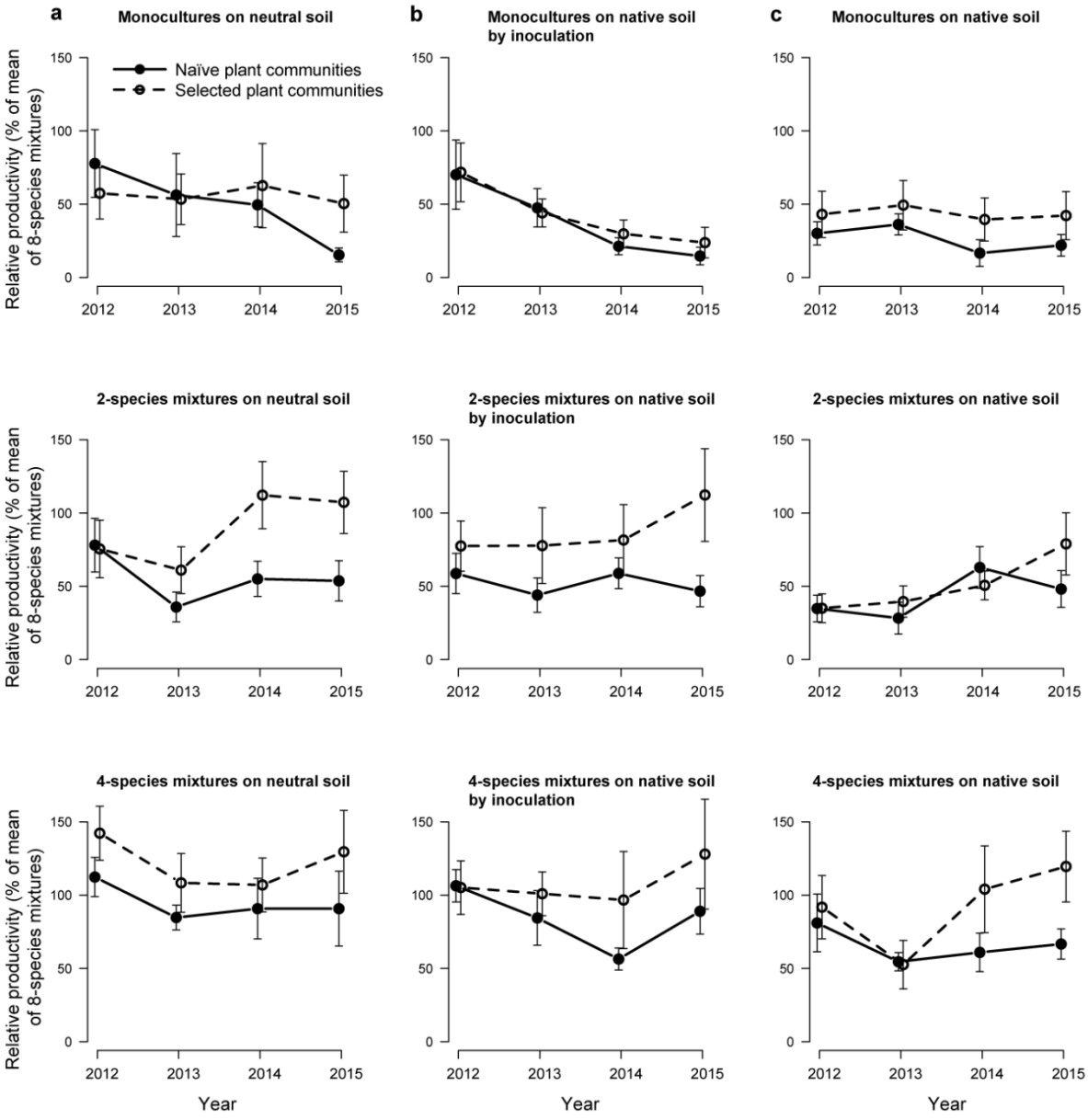

Figure 2 Relative productivity (% of mean of 8-species mixture) of communities of co-selected plants (dashed lines, open circles) and naïve communities (solid lines, closed circles) in monocultures and 2- and 4-species mixtures in **(a)** neutral soil (sterilized soil with neutral inoculum) **(b)** native soil obtained by inoculation (sterilized soil with neutral inoculum and inoculum of co-selected soil biota from original plots) and **(c)** native soil (unsterilized soil with co-selected soil biota from original plots). Raw means and standard errors are shown (for significances see Table S1b).

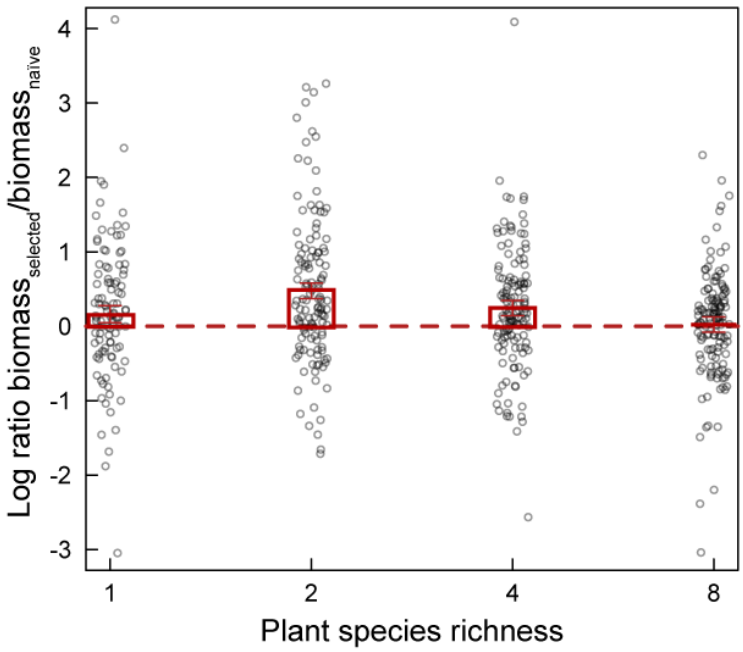

Figure 3 Log ratio of productivity in communities of co-selected plants ($bm_{selected}$) and productivity in naïve communities ($bm_{naïve}$) across years and soil treatments. In 8-species mixtures, productivity did not differ between communities of co-selected and naïve plants (ratio=0). Especially in 2- and 4-species mixtures, but also in monocultures, communities of co-selected plants produced more biomass than naïve communities. Means and standard errors are shown. Raw data are plotted in the background.

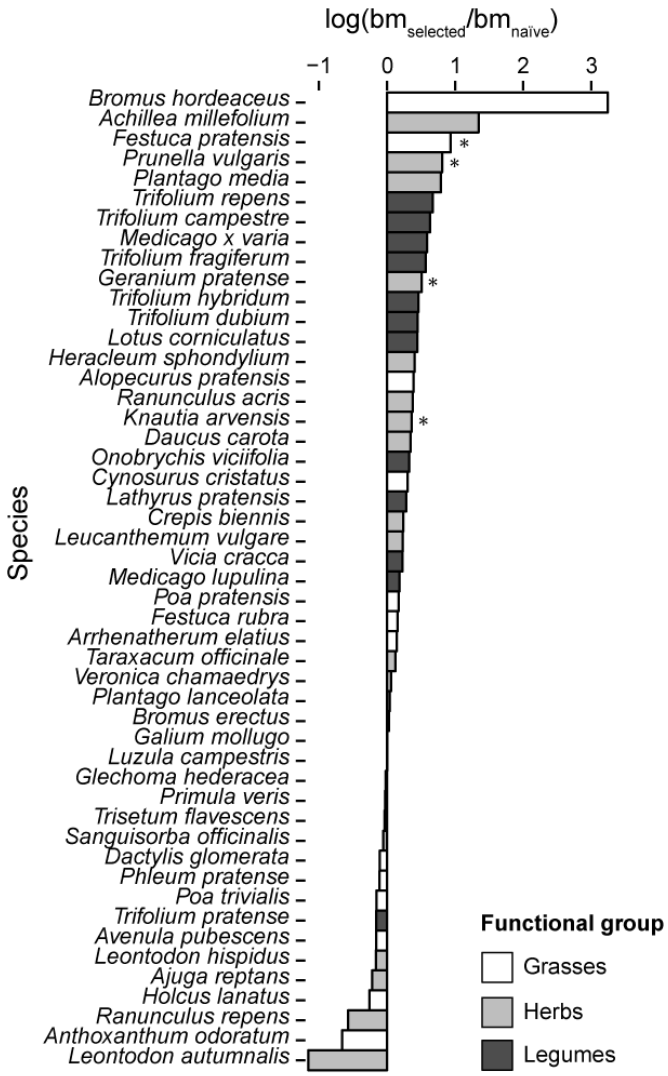

Figure 4 Log-transformed species biomass ratios between co-selected and naïve plants. The majority of plant species attained greater aboveground biomass in communities of co-selected plants compared with naïve communities. The studied plant species belong to three different functional groups: grasses (white bars), herbs (light grey bars) and legumes (dark grey bars). Data are for each species across the four experimental years, across soil treatments and across species richness levels and species compositions of communities (n = 32–352). Three species with n < 32 were excluded from the analysis (*Anthriscus sylvestris*, *Campanula patula* and *Cardamine pratensis*). The stars represent P-values < 0.05 for species tested separately.

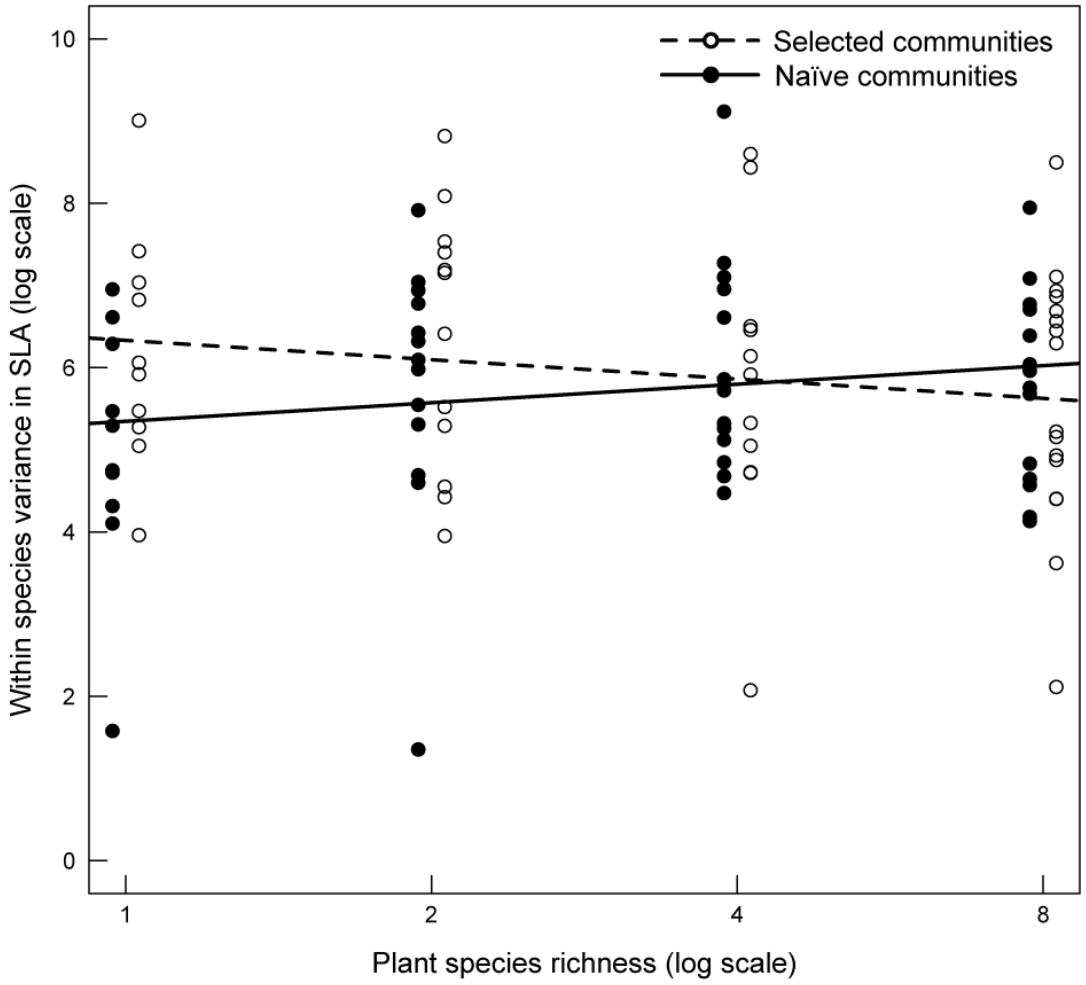

Figure 5 Within-species variation in specific leaf area (SLA) for communities of co-selected plants and naïve communities at the end of the experiment in 2015 in neutral soil. In monocultures within-species variation in SLA (measured as the within-species variance component in analysis of variance) was greater for co-selected than for naïve plants and this difference decreased with increasing species richness. Open circles and dashed line refer to communities of co-selected plants, closed circles and solid line refer to naïve communities. The interaction of log(species richness) and plant history was significant ($F_{1,69.2}$ = 4.87, P = 0.031).

SUPPORTING INFORMATION

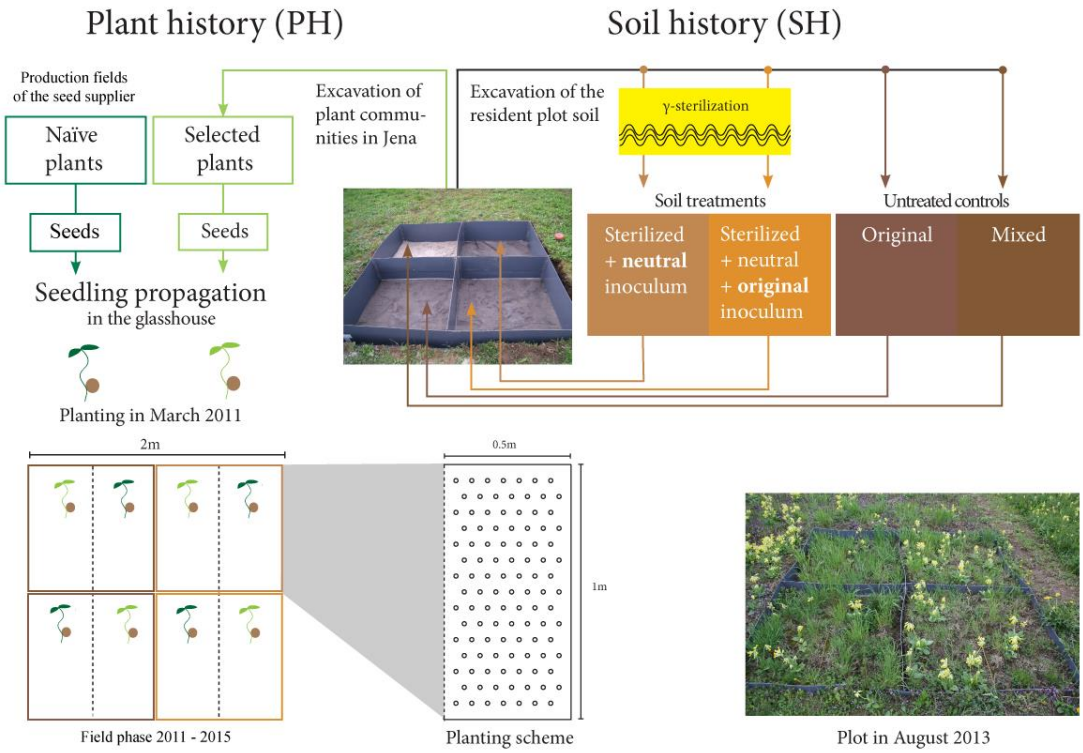

Figure S1 Experimental design. In a glasshouse, co-selected plants were propagated from seeds of plants, which were previously excavated from their communities in the experimental field; naïve plants were propagated from seeds purchased from a seed supplier. Subsequently, the seedlings were planted in the field according to randomized planting schemes with equal species densities. Communities of co-selected plants (light green) and of naïve plants (dark green) were grown in four different soil treatments filled into quadrats (shades of brown), either sterilized or unsterilized, and either containing native soil (with co-selected soil biota) or not. One of the four soil treatments (mixed soil) was forgone after two years of the experiment because the plants were used for a different experiment. Data from this fourth treatment were therefore excluded from all analyses presented in this paper.

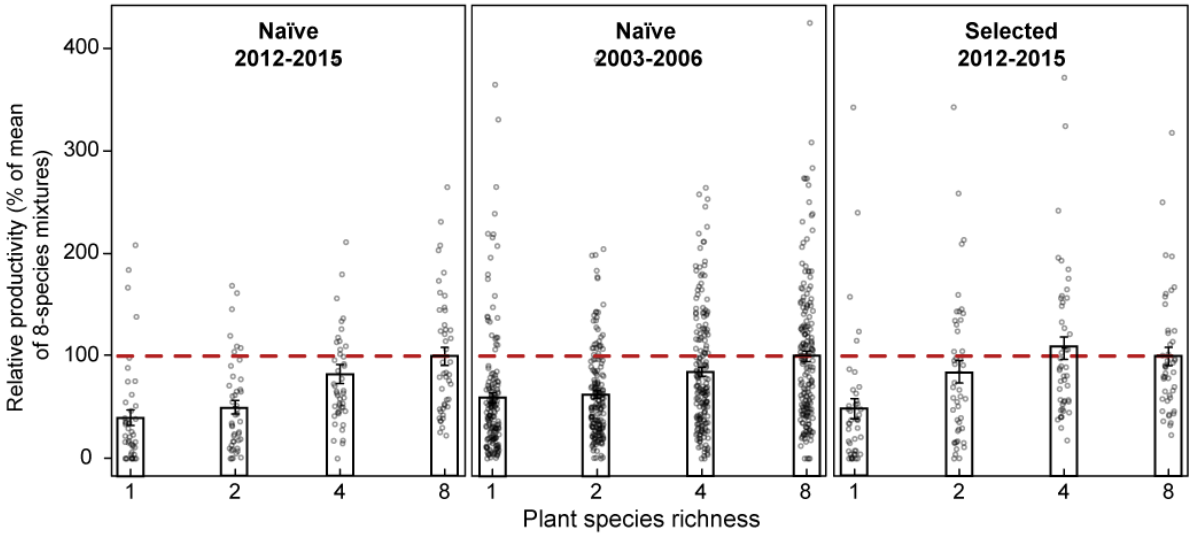

Figure S2 Relative productivity (% of mean 8-species mixture) of naïve plant communities in the current experiment and at the beginning of the Jena Experiment and of communities of co-selected plants, which had been derived from the second type after 8 years of community evolution. The two types of naïve plant communities had similar productivity but were significantly different from the communities of co-selected plants. Means and standard errors of treatments with neutral soil are shown. 100% is indicated by dashed line.

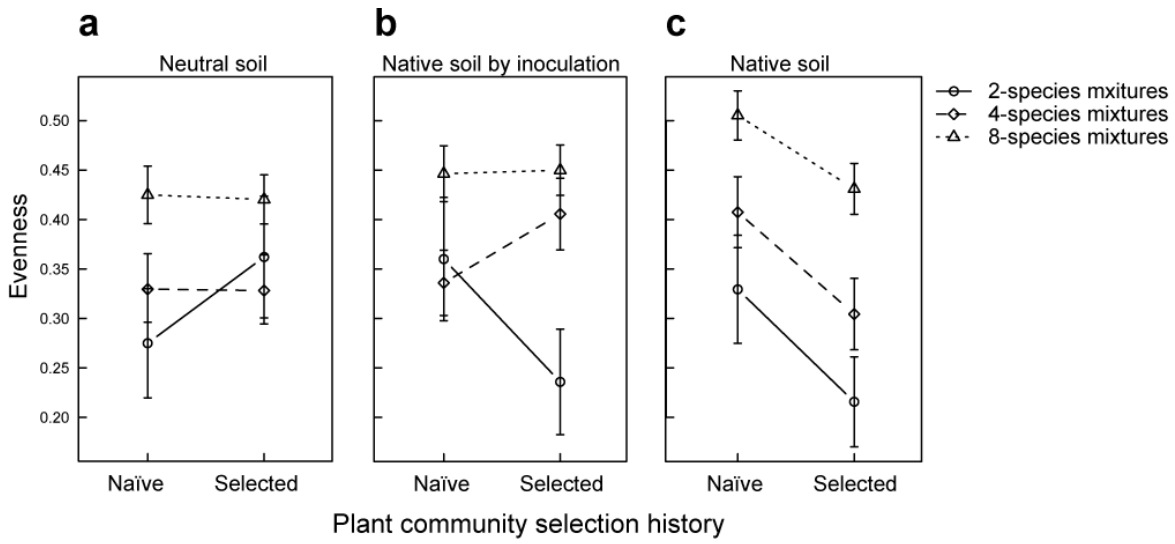

Figure S3 Evenness of naïve communities and communities of co-selected plant species. Evenness was slightly increased in naïve plant communities across all soil treatments (F = 4.088, P = 0.046 for main effect of plant history), which was driven by a much higher evenness for naïve communities in native soil. **a,** Evenness of selected and naïve plant communities in neutral soil obtained by sterilization and inoculation (F = 1.593 and P = 0.209 for effect of plant history). **b,** Evenness of selected and naïve plant communities in native soil obtained by inoculation (inoculum of co-selected microbial communities) (F = 0.360 and P = 0.55 for effect of plant history). **c,** Evenness of selected and naïve plant communities in native soil containing co-selected microbial communities (F = 20.10 and P < 0.001 for effect of plant history). Means and standard errors are shown.

Table S1 Results of mixed-effects ANOVA for the aboveground biomass of the test communities. **(a)** Productivity and **(b)** relative productivity (% of mean productivity of 8-species mixtures per plant history-by-soil treatment-by-year combination).

a	**Productivity**			
Source of variation	**nDf**	**dDF**	***F***	***P***
Factorial species richness (facSR)	3	42.2	7.81	**< 0.001**
Soil history (SH)	2	218.8	16.68	**< 0.001**
facSR × SH	6	218.7	0.64	0.697
Plant history (PH)	1	191.3	23.87	**< 0.001**
facSR × PH	3	191.2	2.77	**0.043**
Factorial harvest (Har)	3	121	1.26	0.290
facSR × Har	9	121	0.99	0.451
SH × Har	6	263.2	4.07	**0.001**
facSR × SH × Har	18	263.2	2.44	**0.001**
PH × Har	3	398.9	2.44	0.064
Variance components	**n**	**Var**	**SE**	**z-ratio**
Plot	47	3707.2	1252.2	2.96
Quadrat	141	0.0	0.0	na
Plot × Har	188	3736.8	762.0	4.90
Half-quadrat	282	2678.8	530.5	5.05
Quadrat × Har	564	1495.1	611.3	2.45
Residual	1128	9046.0	645.7	14.01

b	**Relative productivity**			
Source of variation	**nDf**	**dDF**	***F***	***P***
Factorial species richness (facSR)	3	42.2	8.04	**< 0.001**
Soil history (SH)	2	213.6	16.48	**< 0.001**
facSR × SH	6	213.5	0.53	0.789
Plant history (PH)	1	192.1	16.75	**< 0.001**
facSR × PH	3	191.9	2.90	**0.036**
Factorial harvest (Har)	3	121.1	0.54	0.656
facSR × Har	9	121.1	1.05	0.402
SH × Har	6	263.2	3.91	**0.001**
facSR × SH × Har	18	263.2	2.01	**0.010**
PH × Har	3	401.1	4.23	**0.006**
Variance components	**n**	**Var**	**SE**	**z-ratio**
Plot	47	720.4	240.8	2.99
Quadrat	141	0.0	0.0	na
Plot × Har	188	703.5	144.3	4.87
Half-quadrat	282	496.4	101.7	4.88
Quadrat × Har	564	250.0	117.9	2.12
Residual	1128	1803.1	128.4	14.05

Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, *F* = variance

Table S2 Results of mixed-effects ANOVA for the evenness of selected and naïve plant communities.

	Response: Evenness			
Source of variation	**nDf**	**dDF**	***F***	***P***
Factorial species richness (facSR)	2	33.5	3.74	**0.034**
Soil history (SH)	2	140.4	0.34	0.715
facSR × SH	4	140.4	1.25	0.292
Plant history (PH)	1	132.2	4.28	**0.041**
facSR × PH	2	132.2	0.68	0.508
SH × PH	2	132.2	6.84	**0.001**
facSR × SH × PH	4	132.2	3.43	**0.010**
factorial harvest (Har)	3	99.3	7.29	**< 0.001**
Variance components	**n**	**Var**	**SE**	**z-ratio**
Plot	47	1.00E-02	4.58E-03	2.191
Quadrat	141	4.04E-09	3.23E-10	na
Plot × Har	188	2.28E-02	4.29E-03	5.317
Half-quadrat	282	1.63E-03	1.37E-03	1.189
Quadrat × Har	564	3.98E-03	2.21E-03	1.801
Residual	1128	3.31E-02	2.65E-03	12.486

Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, *F* = variance ratio, *P* = probability of type-I error. Number of replicates (n), variance components (Var) and associated standard errors (SE) for the random effects are provided.

"Factorial species richness" refers to the four diversity levels 1, 2, 4 and 8; "plant history" refers to the community-evolution treatment comparing naïve communities with communities of co-selected plants; "soil history" refers to the three soil treatments and "factorial harvest" refers to the four years 2012–2015.

Table S3 Analysis of similarity (anosim) results for the pairwise comparison of three soil treatments.

Year	Enzyme	Soil comparison	*R*	*P*
2011	Hh	Native vs Neutral	0.307	0.002
2011	Hh	Native vs Native by inoculation	0.258	0.002
2011	Hh	Neutral vs Native by inoculation	0.501	0.472
2011	Taq	Native vs Neutral	0.443	0.002
2011	Taq	Native vs Native by inoculation	0.389	0.002
2011	Taq	Neutral vs Native by inoculation	0.258	0.042
2012	Hh	Native vs Neutral	0.698	0.002
2012	Hh	Native vs Native by inoculation	0.586	0.002
2012	Hh	Neutral vs Native by inoculation	0.389	0.002
2012	Taq	Native vs Neutral	0.627	0.002
2012	Taq	Native vs Native by inoculation	0.501	0.002
2012	Taq	Neutral vs Native by inoculation	0.586	0.006

Note: R = statistic R-value, *P* = significance, number of permutations is 505, calculated with Bray-Curtis dissimilarity. Native soil contained co-selected microbial communities.

Chapter 2:
Testing for co-adaptation of plants and arbuscular mycorrhizal fungi in a biodiversity experiment

Testing for co-adaptation of plants and arbuscular mycorrhizal fungi in a biodiversity experiment

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Authorship

Terhi Hahl, Cameron Wagg, Sofia J. van Moorsel, Debra Zuppinger-Dingley and Bernhard Schmid conceived the study; Terhi Hahl carried out the experiment and collected the data; Terhi Hahl, Bernhard Schmid, Cameron Wagg and Marc W. Schmid analysed the data; Terhi Hahl and Bernhard Schmid wrote the manuscript with other authors contributing to revisions.

ABSTRACT

Interactions between plants and arbuscular mycorrhizal fungi (AMF) have received much attention in the past decades but little evidence exists for potential co-adaptation of plants and AMF in the course of ecological experiments. Here we show that such co-adaptation occurred in a long-term biodiversity experiment in Jena, Germany (the Jena Experiment). Plants selected in monocultures in this biodiversity experiment had evolved positive plant–soil feedbacks after eight years, which could have been due to co-adaptation with AMF and increased pathogen defense. We tested the first possibility as main hypothesis and the second possibility as secondary hypothesis. We conducted a glasshouse plant–soil feedbacks experiment using seven grassland plant species selected over 11 years in monocultures (monoculture-type plants) or mixtures (mixture-type plants). Plants were grown in sterile soil, which we inoculated with corresponding monoculture or mixture AMF from the same plots of the biodiversity experiment, negative control (no AMF) or positive control (*Rhizoglomus irregulare*). We found mixed evidence for co-adaptation between monoculture-type plants and monoculture AMF and between mixture-type plants and mixture AMF; and in the majority of cases the co-adaptation was detrimental rather than beneficial for the plants, indicating a delicate balance where co-adaptation can increase mutualism or parasitism of the specific plant–AMF interaction. Clearer support was obtained for our secondary hypothesis: monoculture-type plants suffered less damage from aboveground pests in the glasshouse, but for the majority of species this came at the cost of reduced growth compared with mixture-type plants. Our results show that co-adaptation between plants and AMF and short-term evolutionary processes can occur in biodiversity experiments but responses to selection in plant monocultures versus mixtures strongly differ between plant functional groups and within them between plant species.

Key words: growth–defense trade-off, plant–AMF co-adaptation, selection, plant–soil feedbacks, rapid evolution

INTRODUCTION

Associations between plants and plant-beneficial or -detrimental soil organisms have received much attention in the past decades (e.g. Bever *et al.*, 1997; Klironomos, 2002; van der Heijden *et al.*, 2006; Petermann *et al.*, 2008; Schnitzer *et al.*, 2011; van der Putten *et al.*, 2013; Van Nuland *et al.*, 2016) but studies have not often considered that interactions between plants and such organisms may alter over ecological time-scales through adaptation (Lekberg & Koide, 2014). In particular, the potential for co-adaptation of plants and beneficial soil organisms in response to local plant diversity has not been tested experimentally so far. Selection in different plant diversities has shown plants are exposed to a stronger accumulation of specialized enemies in monocultures than in diverse plant communities, thus potentially reducing productivity of monocultures over time (Kulmatiski *et al.*, 2012; van der Putten *et al.*, 2013; Marquard *et al.*, 2013). However, a recent study with plants and soils from a biodiversity experiment in Jena, Germany (the Jena Experiment), found that plants in monocultures evolved positive plant–soil feedbacks but plants in mixtures showed negative plant–soil feedbacks after eight years of selection in the respective communities (Zuppinger-Dingley et al. 2016). The assumed selection pressure behind this rapid evolutionary change was on the one hand the mentioned greater accumulation of specialized enemies at low than at high plant species diversity and on the other hand the presence of beneficial soil organisms which could lead to increased mutualistic interactions with plants via co-adaptation. The corresponding hypotheses are that plants selected in monocultures increase defense at the expense of growth potential whereas plants selected in mixtures can trade off reduced defense for increased growth and over time more or stronger mutualistic interactions of plants with soil organisms, here in particular arbuscular mycorrhizal fungi (AMF), can evolve in monocultures or mixtures. To test the second hypothesis, which is the main focus of the present study, reciprocal inoculation experiments (Klironomos, 2002) of plants selected in monocultures or mixtures with AMF selected in the same monocultures or mixtures can be used. If “home” vs. “away” pairings of plants and AMFs affect plants (and in principle also AMF, but this will not be tested here) differently, this can be an indication for co-adaptation of plants and AMF under the particular conditions of the Jena Experiment.

AMF are ubiquitous soil-borne fungi from the division Glomeromycota which form symbiotic relationships with a majority of land plants. By penetrating a root parenchyma of the host plant, the fungus extracts plant-derived carbohydrates that it requires for living (Smith & Smith, 2011). In exchange, the fungus provides mineral nutrients to the host (Gianinazzi-Pearson, 1996; van der Heijden *et al.*, 2006). AMF are able to improve plant survival and growth under certain conditions by increasing nutrient uptake of the host plant (Jones & Smith, 2004; van der Heijden *et al.*, 2006) but also by protecting the plant from the detrimental effects of both above- and belowground enemies (Newsham *et al.*, 1995; Azcón-Aguilar & Barea, 1997; Rodriguez & Redman, 2008; Vannette *et al.*, 2013). Although AMF may promote plant growth, the outcome of the interaction may vary from mutualism to parasitism (Johnson *et al.*, 1997; Klironomos, 2003; Kiers & van der Heijden, 2006; Argüello *et al.*, 2016). Although the dependence of AMF on plant-derived carbon, the short generation time of AMF in comparison to the host plants and limited dispersal (Vályi *et al.*, 2016)

provide the potential for rapid adaptation of AMF (Fenchel & Finlay, 2004; Rúa *et al.*, 2016), studies have rarely considered the possibility that AMF may adapt to the host plants during the short term of ecological experiments (Lekberg & Koide, 2014). On the other hand, studies that have examined evidence for AMF adaptation in comparative field studies without selection imposed by an experimental setting have provided controversial results both for and against AMF adaptation (Weinbaum *et al.*, 1996; Argüello, 2013; Pánková *et al.*, 2014a, 2014b).

Included in the large microbial diversity in rhizosphere (Knief *et al.*, 2012; Lundberg *et al.*, 2012), plants host a variety of pathogenic soil-borne microbes with the ability to reduce plant growth or survival by causing root damages or mortality of seeds and seedlings (Bever *et al.*, 2015). Species-specific soil pathogens have been shown to typically accumulate near the dominant species of the plant community and consequently inhibit the growth of those species (Mordecai, 2011). Similar accumulations have also been found among aboveground pathogens (Rottstock *et al.*, 2014). In monocultures, the accumulation of such specialist pests and the negative effects on plant growth are thus particularly strong, while in diverse plant communities the detrimental effects of specialist pests dilute (van der Putten *et al.*, 2013). In theory, plants may avoid the negative effects of pests by investing resources in defenses (Bezemer & van Dam, 2005) or by improving interactions with beneficial soil organisms, such as AMF (Newsham *et al.*, 1995). In monocultures, plants that allocate more resources to defense or have more beneficial symbioses may, thus, increase genetic representation in the community by improved chances of survival (Bossdorf *et al.*, 2008). In diverse plant communities, on the other hand, interspecific competition rather than pest pressure is more likely to dominate plant selection. Consequently, survival in diverse plant communities may rather depend on the ability of the plant to allocate resources to growth instead of defenses.

Here we conducted a fully reciprocal inoculation experiment to investigate the specific interactions of plants selected for eleven years in monocultures (monoculture-type plants) and species mixtures (mixture-type plants), and AMF communities co-selected with the studied plants for eight plus three years (monoculture AMF and mixture AMF, respectively, with a mixing of soils after the first eight years of selection). Following the co-selection phase, we isolated the AMF communities, allowed their accumulation for ten months, and tested their specific influence on the performance of monoculture- and mixture-type plants in the feedback phase of the experiment. In the feedback phase, we additionally studied the performance of monoculture-type and mixture-type plants in the absence of AMF (control) and in the presence of external AMF, which did not share a common history with the studied plants. We wanted to study whether long-term selection of plants in monocultures vs. mixtures in a biodiversity experiment (the Jena Experiment), led to co-adaptation of plants and AMF. We expected specifically that such co-adaptation would increase the mutualism of plant–AMF associations in home (monoculture-type plants and monoculture AMFs or mixture-type plants and mixture AMFs) compared to away combinations (monoculture-type plants and mixture AMFs or mixture-type plants and monoculture AMFs). In addition, we tested whether monoculture-type plants had been selected for increased defense as a secondary hypothesis

METHODS

Specific associations of plants and co-selected soil microbiota can be studied using reciprocal inoculation experiments (Bever *et al.*, 2015). In such experiments, plants are first grown in identical soil communities to allow the development of plant-specific microbial communities in the rhizosphere (Bever, 1994). If specific microbial groups are of interest, the microbes are isolated afterwards (Klironomos, 2002) and further accumulated in trap cultures to obtain sufficient amount of inoculum for the following phase (Oehl *et al.*, 2003). Finally, an inoculum of soil which includes the trained microbial communities is used in the feedback phase to study the specific influence of the microbial communities on plant performance (Bever, 1994). In the present study, we expanded this design to include a selection phase, in which plants and soil microbes were allowed to co-evolve under different plant diversity treatments in the Jena Experiment, namely monocultures and mixtures.

Plant histories

Our study included seven common perennial European grassland species from four different functional groups: one grass (*Festuca rubra* L.), three small herbs (*Plantago lanceolata* L., *Prunella vulgaris* L. and *Veronica chamaedrys* L.), two tall herbs (*Galium mollugo* L. and *Geranium pratense* L.) and one legume (*Lathyrus pratensis* L.). Each of the studied plant species had undergone 11 years of selection from 2002 until 2014 in either plant monocultures (monoculture-type plants) or species mixtures (mixture-type plants) (Fig. 1, upper part).

First controlled seed production and soil training

After eight years of plant community selection, the plant communities of 48 plots (12 monocultures, 12 two-species mixtures, 12 four-species mixtures and 12 eight-species mixtures) of a biodiversity experiment in Jena, Germany, the Jena Experiment (Roscher *et al.*, 2004), were collected as cuttings in spring 2010 and transplanted in identical plant composition to an experimental garden in Zurich, Switzerland, for the first controlled sexual reproduction among co-selected plants (Zuppinger-Dingley *et al.*, 2014). In addition, the top 30 cm soil of the 48 plots was pooled together, mixed and placed back into the excavated locations in the Jena Experiment. In spring 2011, the seedlings produced from the seeds of the first controlled sexual reproduction in the experimental garden were transplanted back into the mixed soil in the same plots of the Jena Experiment from where the parents had originally been excavated. There, plant communities with identical composition as the original communities were maintained for three years until 2014, to allow them to become re-associated with their own microbial communities.

Second controlled seed production

The seeds used in the present study were obtained from a second controlled sexual reproduction. In March 2014, each entire plant communities from the re-established plots in the Jena Experiment were collected and established in their respective communities in plots in the experimental garden in Zurich. For out study, we collected seeds from seven monoculture plots, one four-species mixture plot and six eight-species mixture 1x1 m plots in

the experimental garden. The plots were filled with 30 cm of soil (1:1 mixture of garden compost and field soil, pH 7.4, commercial name Gartenhumus, RICOTER Erdaufbereitung AG, Aarberg, Switzerland), and fenced with netting to minimize cross-pollination with plants outside the plots. The seeds of the seven plant species were stored at +4 ˚C for at least two months.

Soil collection and inoculum preparation

In March 2014, we collected rhizosphere soil samples attached to the roots of the plants which we transported to Zurich for the second sexual reproduction event (Fig. 1, fourth row from bottom). Thus, by the time of our soil sampling, the soil communities had undergone three years of community assembly and eight plus three years of potential co-evolution with each of the seven plant species in monocultures (monoculture-type plants) or mixtures (mixture-type plants).

To isolate AMF communities from the sampled rhizosphere soils, we passed deionized water and 25 g of soil sample through a series of sieves, isolated soil particles with a diameter of 32–500 µm using a sugar gradient-centrifugation method (Sieverding, 1991), and finally collected the AMF spores manually with a pipet under a microscope at 200-fold magnification. To allow accumulation of the isolated AMF communities, we established trap cultures that consisted of 2 L of 4:1 sand-soil mixture, autoclaved at 120 °C for 99 min, and a monoculture of trap plants of one each of the seven tested plant species (Fig. 1, second row from bottom). All trap cultures received 300–400 AMF spores in 30 ml of deionized water, except for the negative control trap cultures, which received 30 ml of deionized water without AMF spores. For the trap plants we used seeds from a commercial seed supplier, which provided the original seed material for the Jena Experiment (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany). To avoid contamination of the trap cultures, the seeds were surface-sterilized with 7–14 % bleach for 10–45 min and afterwards pre-germinated on 1% water agar. We deliberately avoided that the trap plants shared a "community-selection" history (van Moorsel *et al.*, 2017) with the AMF spores collected from the rhizosphere of monoculture- or mixture-type plants of the same species. Each AMF trap culture existed in two replicates. After ten months of growth in the glasshouse, we collected a root sample from each trap culture, fixed the root samples in 50 % ethanol, cleared them with 10 % KOH, stained them with 5 % ink-vinegar (Vierheilig *et al.*, 1998), and quantified the AMF colonization microscopically. From trap culture pots in which fungal colonization was detected, we further quantified the concentration of AMF spores by collecting a 10-g soil sample, isolating AMF spores with the same sieving and centrifugation methods used when setting up the AMF trap-culture pots, and counted the AMF spores under a microscope. Only five of the seven plant species in the two replicates had sufficient AMF colonization for both monoculture- and mixture-AMF communities. Trap cultures that showed fungal root colonization were dried and the plants were harvested at ground level, the roots were harvested and cut into 3–5 cm fragments and the belowground content of the trap cultures was used as soil inoculum in the plant–soil feedback experiment described below.

For the positive control-soil treatment, we used a trap culture substrate containing *Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl as the inoculum. We developed the culture for nine months in a substrate of 15 % soil, 65 %

sand and 20 % oil binder with *Plantago lanceolata* which had no shared community-selection history with plants or soils from the Jena Experiment. *R. irregulare* (previous names *Glomus intraradices* and *Rhizophagus irregulare*; (Sieverding *et al.*, 2015)) is an AMF taxon common in natural grasslands. The *R. irregulare* material we used in the present study was obtained from M.G.A. van der Heijden's Ecological Farming Group (Agroscope Reckenholz Tänikon, Zurich, Switzerland).

Assessment of soil N and P content at the beginning of the experiment

We conducted Olsen-P and N-mineralization analyses to confirm that the content of phosphate and ammonium, respectively, did not vary in the inoculated substrate at the beginning of the experiment. For Olsen-P analysis, phosphorus was extracted from a 2 g soil sample following the procedure of Olsen *et al.* (1954). We incubated 20 g of soil sample at 40 °C for 7 days in waterlogged conditions for N-mineralization (Keeney, 1982) and then extracted ammonium with 2M KCl (Kandeler & Gerber, 1988). The extracted phosphorus and nitrogen content was measured using the San^{++} Continuous Flow Analyzer (Skalar Analytical B.V., Breda, The Netherlands). The inoculated experimental substrate had a phosphate content of 3.76 mg kg^{-1} and an ammonium content of 4.58 mg kg^{-1}, which did not vary among the inoculum treatments (soil P: $F_{4, 17} = 1.53$, $P = 0.238$ and soil N: $F_{4, 17} = 1.53$, $P = 0.239$).

Setup of plant–soil feedback experiment

To establish the soil treatments of the present study, we filled 1-L pots with gamma-radiated (27–54 kGy) 1:1 (weight/weight) sand-soil mixture and added 9 % (volume/volume) of inoculum without AMF (control), inoculum of AMF isolated from plants grown in monoculture (monoculture AMF) or mixture (mixture AMF), or inoculum containing *Rhizoglomus irregulare.* One monoculture- or mixture-type plant of a single test species was planted in each pot (Fig. 1, bottom row). To standardize the non-AMF microbial community within each pot, we created a microbial wash by filtering 1.2 L of a mixture of unsterilized field soil and the AMF trap culture substrates through a series of sieves and finally through MN615 (Macherey-Nagel GmbH & Co. KG) filter paper with 5 L of deionized water and confirmed the absence of AMF spores in the filtrate microscopically. Each pot received 10 ml of the microbial-wash filtrate. The experiment included four soil treatments in total, two plant histories (monoculture- and mixture-type plants) and seven plant species in a full factorial design (Table 1). Three species without sufficient AMF colonization in the trap cultures were grown only in the control and *R. irregulare* soil treatments. Combinations of these two soil treatments were replicated five times, and the two other AMF treatments were replicated ten times (five times per trap-culture replicate). The 337 pots were randomly arranged within five experimental blocks in a glasshouse compartment with each particular treatment combination and trap-culture replicate occurring only once in each block.

Seed and seedling mortality

Seeds collected from *G. mollugo* mixture-type plants repeatedly developed mould while germinating on the agar plates. As a consequence of the low germination rate of mixture-type *G. mollugo*, the experiment included three *G. mollugo* mixture-type plants less than

monoculture-type plants (Table 1). At the beginning of the experiment, we observed fungus gnats (*Bradysia* spp.). This was the cause of some of the plant mortality during the experiment.

Data collection

We harvested plant aboveground biomass for the first time three months after planting seedlings into the pots of the different soil treatments, cutting the plants to 4 cm aboveground. At the second and final harvest, after five months of plant growth, maximum height and average leaf absorbance (SPAD-502Plus Chlorophyll Meter, KONICA MINOLTA, INC., Osaka, Japan) of three representative leaves of each plant were measured and the aboveground biomass harvested at ground-level (Table 2). Leaf absorbance of *F. rubra* was not measured because the leaves were too narrow. The biomass of each plant was dried at 70 °C for 48 h and then weighed. We assessed leaf mass per area (LMA) and leaf dry matter content (LDMC) at the second harvest by measuring the area of fresh leaves (LI-3100C Area Meter, LI-COR, Lincoln, USA) immediately after harvest and assessing the weight of the leaves before (fresh weight) and after drying (dry weight) (Table 2). Finally, we estimated the degree of pest damage on plant aboveground tissues (Table 2) due to powdery mildew (family Erysiphaceae) and two-spotted spider mites (*Tetranychus urticae* Koch) in the glasshouse. To determine the AMF colonization of plant roots at the end of the experiment, roots and adhering rhizosphere soil were cut into small fragments and random subsamples of roots were then stored in 50 % ethanol for microscopic quantification of AMF using the same clearing and staining method as described above (Vierheilig *et al.* 1998).

Data analysis

We analysed the data of the two aboveground biomass harvests, morphological trait measurements, leaf damage estimates and AMF colonization rate using linear models, and the data of plant survival and AMF colonization probability using analysis of deviance, and summarized the results in analysis of variance (ANOVA) and deviance (ANDEV) tables, respectively (McCullagh & Nelder, 1998; Schmid *et al.*, 2017). The explanatory terms of the models were block, plant functional group, species identity within plant functional group, plant history (monoculture-type vs. mixture-type), soil treatments (four soil treatments or sequence of the following three orthogonal contrasts: control vs. AMF treatments, *R. irregulare* vs. monoculture or mixture AMF and monoculture vs. mixture AMF) and interactions of these. Statistical analyses were conducted using the software product R, version 3.0.2 (R Core Team, 2013).

RESULTS

Plant survival

Of the 337 studied plants, 259 plants survived at the end of the experiment with a survival rate of 77 %. Plant survival differed significantly between functional groups and species within functional groups (Table 3, Fig. 2). Mixture-type plants had on average significantly higher survival than monoculture-type plants with the exception of *G. mollugo* ($P = 0.012$ for

main effect of plant history after exclusion of *G. mollugo*). The lowest observed plant survival occurred in control soil, suggesting that the presence of AMF increased the plants chance of survival. We observed the highest survival in soils containing *R. irregulare* inoculum among the AMF treatments. There were no overall differences between monoculture and mixture AMF and no indication that monoculture-type plants survived better with monoculture AMF or mixture-type plants with mixture AMF. However, there was a significant interaction between plant functional group and monoculture vs. mixture AMF: mixture AMF improved the survival of herb plants whereas monoculture AMF improved survival of the legume *L. pratensis*.

Plant biomass production

Aboveground biomass production differed significantly between plant functional groups and between species within functional groups at both harvests (Tables 4–5, Figs 3–4). At the first harvest, mixture-type plants of four species *P. lanceolata*, *P. vulgaris*, *V. chamaedrys* and *G. pratense* had more biomass than monoculture-type plants whereas the opposite was true for the species *F. rubra*, *L. pratensis* and *G. mollugo*. The difference in biomass production between monoculture- and mixture-type plants was smaller at the second harvest but still varied significantly among the different plant functional groups. The majority of plant species produced lowest aboveground biomass at both harvests in control soil and the beneficial effect of AMF was even more obvious when only those plants for which AMF colonization of roots was detected were included among the three AMF soil treatments (compare Fig. 3C and 4C with Fig. 3A and 4A, respectively). Only *V. chamaedrys* mixture-type plants at the first harvest and *V. chamaedrys* of both monoculture- and mixture-type plants at the second harvest produced more biomass in control soil than in the AMF-inoculated soil treatments (Table 4 and Fig. 3B, Table 5 and Fig. 4B, respectively). *Rhizoglomus irregulare* significantly increased biomass production compared with monoculture and mixture AMF at the first harvest but reduced biomass marginally at the second harvest (Table 5, Figs 4A and 4C).

AMF presence and AMF colonization rate

We observed AMF colonization in 77 % of the plants that survived until the end of the experiment in the AMF-inoculated soil treatments (Fig. 5). Plant functional group and species identity explained the variation in AMF colonization probability (Table 6) and in AMF colonization rate (Table 7, Fig. 6). *Veronica chamaedrys* had the lowest AMF colonization, which mirrored its lower biomass production in AMF-inoculated than in control soil. As expected, AMF colonization was practically absent in the control-soil treatment, indicating that contamination of pots with AMF spores from outside was very unlikely. Better AMF colonization than with monoculture or mixture AMF was obtained in the "positive control" soil treatment with *R. irregulare* inoculum. AMF colonization probability with *R. irregulare* was higher for monoculture- than for mixture-type plants and, surprisingly, tended to be lower for the "home" combinations of monoculture-type plants with monoculture AMF and mixture-type plants with mixture AMF than for "away" combinations of monoculture-type plants with mixture AMF and mixture-type plants with monoculture AMF and this was

particularly clear for *L. pratensis*, the representative of the legume functional group (P = 0.039 for "PH x ST", P = 0.086 for "PH x F" and P = 0.022 for "FG x PH x F" in Table 6).

Monoculture AMF gave higher root colonization rates than mixture AMF in the species representing legumes (*L. pratensis*) and tall herbs (*G. mollugo*) but mixture AMF gave higher root colonization than monoculture AMF in the species representing small herbs (*P. lanceolata*, *P. vulgaris* and *V. chamaedrys*; P = 0.009 in Table 7). Inoculation of soil by *R. irregulare* produced higher root colonization rates than monoculture or mixture AMF (Fig. 6) but in this soil treatment colonization rate was not well correlated with plant biomass production (Fig 7). Root colonization rate was positively correlated with biomass production, howerver, for plants growing in soil inoculated with monoculture or mixture AMF.

Leaf damage

Mixture-type plants were on average more severely damaged than monoculture-type plants by the common pests affecting the plants in the glasshouse (see "Methods") and this effect was particularly strong in *P. lanceolata* (Table 8, Fig. 8). Mixture-type plants of *P. lanceolata* had severe powdery mildew infections (Fig 8).

Plant traits

All measured plant traits differed significantly between plant functional group and species within functional group (Tables S1–S4, Figs S1–S4). Mixture-type plants had higher LDMC than monoculture-type plants of *P. lanceolata*, *P. vulgaris*, *L. pratensis* and *G. pratense* whereas the opposite was the case for *V. chamaedrys* and *G. mollugo* (P = 0.037 in Table S1; Fig. S1). Similarly, mixture-type plants of *P. lanceolata*, *P. vulgaris*, *L. pratensis*, *G. mollugo* and *G. pratense* had higher LMA than monoculture-type plants whereas the opposite was the case for *F. rubra* and *V. chamaedrys* (P = 0.041 in Table S2; Fig. S2). In addition, monoculture-type plants were generally taller than mixture-type plants, with the exception of the legume *L. pratensis* and the small herb *P. vulgaris* (P = 0.02 for "PH" and P < 0.001 for "FG x PH" in Table S3; Fig. S3).

Soil treatments alone did not explain variation in plant traits, but in contrast to the analyses of survival, biomass and AMF colonization, there were more indications for interactions between plant functional groups ("FG") or species ("SP"), plant history ("PH") and the soil contrast monoculture vs. mixture AMF ("F"). Of particular interest are those interactions involving the one-degree-of-freedom term "PH x F", because this provides a test for co-adaptation between plants and AMF grown in monoculture vs. mixture, i.e. if home-combinations of monoculture-type plants with monoculture AMF and mixture-type plants with mixture AMF differ from away-combinations monoculture-type plants with mixture AMF and mixture-type plants with monoculture AMF. Co-selected AMF increased leaf absorbance in the small herbs *P. lanceolata*, *P. vulgaris* and *V. chamaedrys* whereas the opposite was the case for the legume *L. pratensis* and the tall herb *G. mollugo* (P = 0.002 in Table S4, Fig. S4). Co-selected AMF also increased LMA of mixture-type plants of *P. lanceolata* and *G. mollugo*, monoculture-type plants of *L. pratensis* and both plant histories of *P. vulgaris* whereas the opposite was observed in mixture-type plants of *L. pratensis* and both plant histories of *V. chamaedrys* (P < 0.001 in Table S2, Fig. S2). In addition, the two representatives of the legume (*L. pratensis)* and the tall herb (*G. mollugo)* functional groups

tended to grow taller with mixture than with monoculture AMF, which was not the case for the small herbs ($P < 0.001$ in Table S3, Fig. S3).

DISCUSSION

In the present study we hypothesized that combinations of plants and AMF co-selected in monocultures or in mixtures would be "beneficial" to plants compared with combinations of the same plants and AMF without shared selection history. In particular, based on earlier results (Zuppinger-Dingley et al. 2016), we expected that monoculture-type plants may have been selected for increased beneficial associations with AMF and improved defense against enemies potentially accumulating over time in plant monocultures. We tested our hypothesis with seven plant species belonging to four different functional groups.

AMF-inoculated soil treatments in this study showed colonization of plant roots, confirming that the AMF spore isolation from the field soil, the subsequent AMF trap-culturing and the inoculation of the soil treatments was successful. However, in approximately a fifth of the plants that had been growing in AMF-inoculated soils, root colonization was not visible at the second harvest. The apparent absence of colonization may in part be explained by our estimated AMF colonization from a random sub-sample of roots rather than of the entire root system as plants may have been colonized elsewhere in the root system or roots with AMF may have died during the course of this experiment. As we could not identify which plants were false negatives for AMF colonization, we conducted separate analyses across all plants of the present study and across plants which were visibly colonized by AMF at harvest.

Although the colonization probability and colonization rate of AMF varied between plant functional groups and species, there was no indication that monoculture AMF associated more intensively with monoculture-type plants and mixture AMF with mixture-type plants. In fact, there was an indication that the opposite was the case with regard to colonization probability (see Fig. 5A); and monoculture AMF had higher colonization rates than mixture AMF in legumes and tall herbs but not in small herbs (see Fig. 6B). Furthermore, we did not observe any difference in the survival of monoculture- or mixture-type plants in response to monoculture or mixture AMF and both monoculture and mixture AMF similarly improved biomass production of monoculture- and mixture-type plants (see Fig. 7). However, there was some indication of co-adaptation between monoculture-type plants and monoculture AMF and between mixture-type plants and mixture AMF (home combinations) with respect to plant traits. Namely, co-selected AMF increased leaf absorbance and LMA in three and two small herb species, respectively, but the opposite response was observed for leaf absorbance in representatives of legumes and tall herbs and for LMA in one small herb species (see Figs S1, S2). Increased leaf absorbance and high LMA are related to higher area-based nitrogen content (Niinemets, 1997; Moran *et al.*, 2000) suggesting that co-selected AMF may have improved the nitrogen uptake of at least two of the small herbs, whereas small herb *V. chamaedrys* generally showed decreased performance when grown in the presence of AMF. In a previous study of two species of *Prunella*, strong effects were found with of co-occurring AMF on plant aboveground morphological traits

(Streitwolf-Engel *et al.*, 1997). In our study, we found that AMF which differed only in their selection history in plant monoculture- vs. mixture, had similar effects on several of the tested plant species.

Our results did not in general support our hypothesis that co-selection of plants and AMF in plant monocultures or mixtures leads to more beneficial associations between plants and AMF, and sometimes even the opposite was observed, suggesting that the tendency for co-adaptation with AMF may vary between plant functional groups and species, leading to beneficial, neutral or even detrimental effects for the plant partner in the association. It is conceivable that the results may be different for the fungal partner than for the colonized plant (Argüello *et al.*, 2016), but this was not tested here. Previous studies examining the co-adaptation of AMF and plants have found variable results ranging from those supporting co-adaptation (Weinbaum *et al.*, 1996; Argüello, 2013; Pánková *et al.*, 2014a) to those that do not (Pánková *et al.*, 2014b). A recent meta-analysis (Rúa *et al.*, 2016) suggested that the variability in the outcomes of such studies is influenced by the origin of the soil in which the co-adaptation is tested, as chances to find evidence for co-adaptation were higher when plant, soil and AMF shared a common origin. The present results do not support this suggestion as eight plus three years of co-selection of plants and AMF did not generally result in more beneficial associations. Although we mixed soils after the first eight years of co-selection, the AMF had the potential to re-associate with "their" plant species and continue to co-evolve with them during the second three years of co-selection. The novel feature of our study was that after first isolating monoculture- and mixture-type plants and monoculture and mixture AMF, we grew them again in association with each other in our glasshouse experiment. It is conceivable that other factors may have resulted in the beneficial effects of a common plant and soil history in previous studies included in Rúa *et al.* (2016) and the one of Zuppinger-Dingley *et al.* (2016).

In addition to plant-beneficial or -detrimental co-adaptation between plants and AMFs in our experiment mentioned above, there were significant differences between the AMF collected from the Jena Experiment and the "control" AMF *R. irregulare*, which did not share a common history with the experimental plants. Interestingly, *R. irregulare* colonization was greater than mixture or monoculture AMF and led to higher initial plant biomass, but at the second harvest its effect on plant biomass was marginally negative and its colonization rate did not show a clear correlation with plant biomass (see Fig. 7). The higher colonization rate of *R. irregulare* compared to monoculture or mixture AMF could have associated with a greater carbon demand and reduced the amount of resources available for the plant re-growth after the first harvest. In addition to the lack of common history, the soil treatment with *R. irregulare* differed from the other AMF treatments with respect to AMF diversity: inoculum with *R. irregulare* represented a single AMF species whereas monoculture and mixture AMF inocula likely included several AMF species. As increasing AMF diversity may stabilize the outcome of plant–AMF symbiosis (van der Heijden *et al.*, 1998), the more positive effect of AMF colonization on final plant biomass with the monoculture and mixture AMF than with *R. irregulare* might in part have been also caused by AMF diversity effects. A high colonization ability of *R. irregulare* has similarly been observed in previous studies (e.g. Engelmoer *et al.*, 2014), however the high rate of colonization in the present study may additionally be explained by the absence of AMF

competitors in the *R. irregulare* soil inoculum, as competition between AMF species tends to reduce the overall success of AMF colonization (Engelmoer *et al.*, 2014).

Mixture-type plants of all three species of small herbs and the tall herb *G. pratense* showed greater average biomass production than monoculture-type plants at the first harvest, but the opposite was the case for the tall herb *G. mollugo*, the grass *F. rubra* and the legume *L. pratense*. Such differences in biomass production between monoculture- and mixture-type plants were smaller at the second harvest but still significant between functional groups. Out of the seven species of the present study, four species showed higher LDMC and five species higher LMA for mixture-type plants in comparison to monoculture-type plants, suggesting that these mixture-type plants invested more resources into leaf biomass production than monoculture-type plants. The accumulation of specialized pests is a well-known phenomenon in monocultures, which may drive differential selection of plants at low vs. high species diversity (Zuppinger-Dingley *et al.*, 2016). We hypothesized that monoculture-type plants may have been selected for improved pest defense at the cost of reduced growth potential. Because specialized pests tend to dilute in diverse plant communities (Eisenhauer *et al.*, 2012), we expected mixture-type plants to not trade off their growth potential to increase defense against pests. The increase in biomass production of mixture-type plants in comparison to monoculture-type plants for four of our seven plant species supported this hypothesis but the opposite result for the three other species did not. However, the hypothesis that monoculture-type plants should evolve increased defense was generally confirmed when pests appeared in the glasshouse and caused greater leaf damage in mixture-type plants (see Fig. 8). We observed particularly severe infections by the fungal pathogen powdery mildew in mixture-type plants of *P. lanceolata*, suggesting, in agreement with Engelmoer et al. (2014), that monoculture-type plants of *P. lanceolata* may have been subjected to particularly strong selection pressure for pathogen defense in comparison with mixture-type plants.

Mixture-type plants also showed greater survival than monoculture-type plants in the present study. Some plant mortality in the present experiment was due to pests, particularly from the larvae of fungus gnats which fed on plant root tissue. We did not record the extent of the belowground damage and recorded only the amount of leaf damage at the end of the experiment of surviving plants. Thus, we cannot confirm whether the reduced monoculture-type plant survival was due to increased pest damage, however, we would not expect this in the light of the hypothesis that monoculture-type plants should be better defended than mixture-type plants. It is also unclear whether AMF colonization played a role in the differential survival between monoculture- and mixture type plants as we estimated colonization probability and rate of AMF only in the surviving plants. Considering that the amount of leaf damage rates did not differ between control and AMF-inoculated soil treatments, the observed lower survival of monoculture-type plants coupled with lower pest damage in the present study suggests those monoculture-type plants that survived the experiment were better defended than the plants that died.

Conclusions

In the present study, we found limited evidence for co-adaptation of plants and AMF after eight plus three years of “co”-selection in plant monocultures vs. mixtures. Furthermore, in those cases where we did find co-adaptation it was often detrimental to the plant, our

results did not support the hypothesis that monoculture-or mixture-type plants may be selected for more beneficial mutualism with their home AMF, i.e. monoculture or mixture AMF, respectively. This suggests that co-adaptation between plants and AMF in plant biodiversity experiments does not follow a general pattern leading to increased mutualism but rather depends on the specificity of the context and more resembles an arms race where sometimes the outcome may be reduced mutualism, depending on the plant functional group or species involved. However, we did find consistent evidence that surviving monoculture-type plants may have been selected for improved defense, potentially in response to an accumulation of specialized pests in monocultures over time. Monoculture-type plants died more often than mixture-type plants in the present study suggesting that the monoculture-type plants that died during the experiment were potentially more vulnerable to pests than the monoculture-type plants that survived. Here we examined the potential co-adaptation of AMF and monoculture- vs. mixture-type plants to disentangle the mechanisms underlying the previously observed evolution of positive plant–soil feedbacks among monoculture-type plants in contrast to mixture-type plants in biodiversity experiments (Zuppinger-Dingley *et al.*, 2016). From the present study, we conclude that other beneficial soil organisms or increased defenses against monoculture- but not mixture-specific pests might have caused these previously observed effects. Finally, the lower defense potential of mixture- in comparison with monoculture-type plants offers a potential explanation for the previously observed negative plant–soil feedbacks in mixture-type plants.

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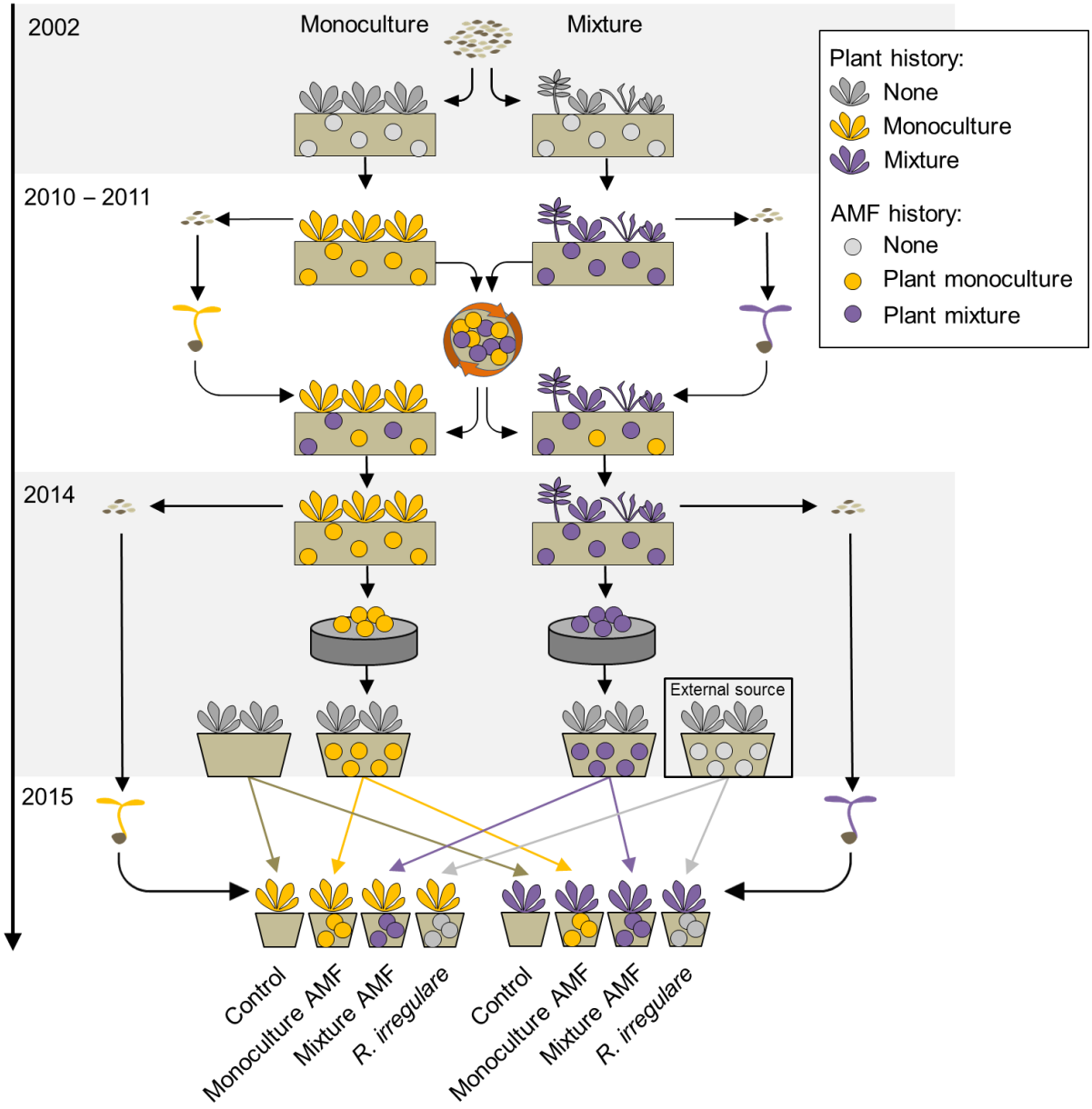
2002
Monoculture
Mixture
2010 – 2011
2014
2015
External source
Plant history:
None
Monoculture
Mixture
AMF history:
None
Plant monoculture
Plant mixture
Control
Monoculture AMF
Mixture AMF
R. irregulare
Control
Monoculture AMF
Mixture AMF
R. irregulare

Figure 1. Experimental design. Plant monocultures and mixtures were sown in 2002 in the Jena Experiment and the communities were maintained until 2010. During this time, plants and soil microbial communities shared a common selection history and could potentially co-evolve. In 2010, plants of 48 plots were taken to the first controlled seed production event, and the soil of the plots was pooled, mixed and placed back to the excavated locations. In spring 2011, the seedlings produced from the first seed production event were transplanted back to the mixed soil in the same plots from where their parents had been excavated. The plant communities were allowed to become associated with their own microbial communities until year 2014. During this time, plants and soli microbial communities could again potentially co-assemble and co-evolve. In spring 2014, plants were taken to the second controlled seed production event, and the AMF spores from their rhizosphere soil were isolated. The isolated AMF communities were allowed to accumulate in trap-cultures for 10 months with trap plants that did not share a community-selection history with the AMF spores. For negative control, we also produced similar control trap-cultures without AMF spores. To establish soil treatments of the present study we filled pots with sterile soil and added 9 % inoculum without AMF (control), inoculum of AMF isolated from plants grown in monoculture (monoculture AMF) or mixture (mixture AMF) or inoculum containing externally produced *Rhizoglomus irregulare*. Finally, the plants with the different selection histories in monoculture (monoculture-type plants) and mixture (mixture-type plants) were separately planted into these pots with the different soil treatments. Our main hypothesis was that combinations of monoculture-type plants with monoculture AMF and mixture-type plants with mixture AMF would be "beneficial". Despite significant effects of plant-history and soil treatments we found only limited support for the hypothesis in some of the seven plant species tested.

Table 1. Number of replicates for monoculture- and mixture-type plants of seven plant species grown on the four soil treatments of the experiment.

		Soil treatments			
Species	Plant history	Control	Monoculture AMF	Mixture AMF	*R. irregulare*
Festuca rubra	Monoculture	5	0	0	5
	Mixture	5	0	0	5
Plantago lanceolata	Monoculture	5	10	10	5
	Mixture	5	10	10	5
Prunella vulgaris	Monoculture	5	10	10	5
	Mixture	5	10	10	5
Veronica chamaedrys	Monoculture	5	10	10	5
	Mixture	5	10	10	5
Lathyrus pratensis	Monoculture	5	10	10	5
	Mixture	5	10	10	5
Galium mollugo	Monoculture	5	10	10	5
	Mixture	5	9	8	5
Geranium pratense	Monoculture	5	0	0	5
	Mixture	5	0	0	5
				N = 337	

Table 2. Plant traits measured during the experiment at the given time points.

Measured plant trait	Unit	Plant age (weeks)
Aboveground biomass, first harvest	g dry weight/pot	12
Aboveground biomass, second harvest	g dry weight/pot	20
Leaf damage	rate 0–5 (none to high)	20
AMF colonization probability	presence/absence	20
AMF colonization rate	%	20
Leaf absorbance	SPAD (0-50)	19
Leaf dry matter content (LDMC)	mg dry weight g^{-1} fresh weight	20
Leaf mass per area (LMA)	g dry weight/cm^2	20
Maximum height	cm	19

Note: SPAD values are index values, defined by the manufacturer of the chlorophyll content measuring device, that indicate the relative amount of chlorophyll present in the leaf.

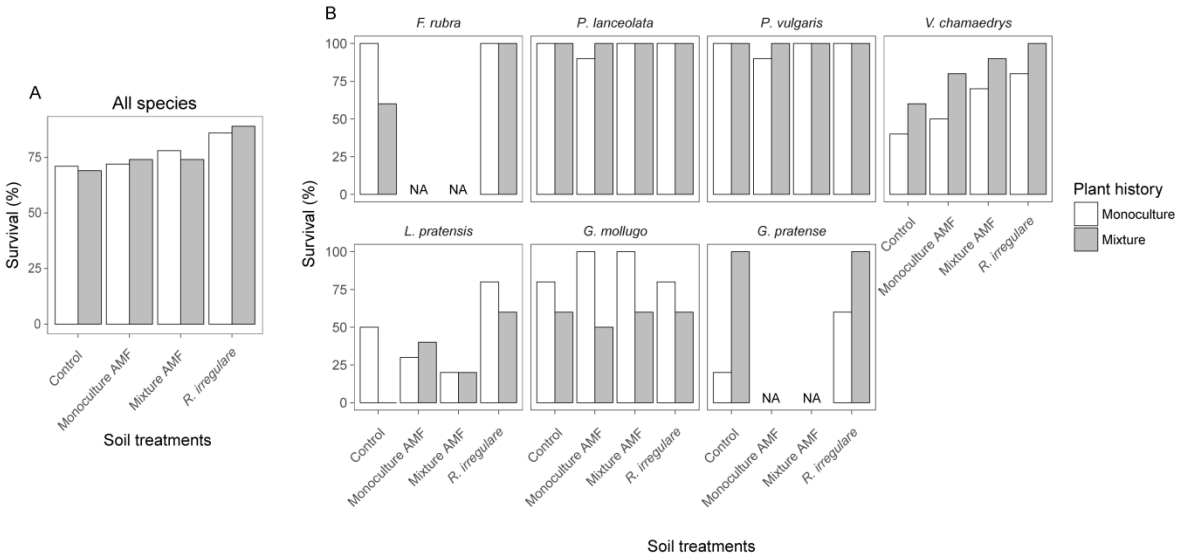

Figure 2. Survival of monoculture- (white bars) and mixture-type plants (grey bars) in the four soil treatments: **A**, across all species; **B**, by species. Bars are proportions of survivors out of all planted individuals in the experiment.

Table 3. Analysis of deviance (ANDEV) for plant survival.

Source of variation	Df	%-DV	*P*	
Block	4	0.7	0.436	
Functional group (FG)	3	18.5	<0.001	***
Species within FG (SP)	3	8.0	<0.001	***
Plant history (PH)	1	0.0	0.633	
Soil treatment (ST)	3	2.6	0.002	**
Control vs. AMF treatments (C)	1	1.3	0.008	**
R. irregulare vs. monoculture or mixture AMF (R)	1	1.1	0.015	*
Monoculture vs. mixture AMF (F)	1	0.2	0.253	
FG × PH	3	2.7	0.003	**
FG × ST	7	3.0	0.018	*
FG × C	3	0.4	0.593	
FG × R	2	1.5	0.017	*
FG × F	2	1.2	0.043	*
SP × PH	3	5.1	<0.001	***
Residuals	308	53.7		

Notes: Df, degrees of freedom; %-DV, proportion of total deviance; *P*, error probability

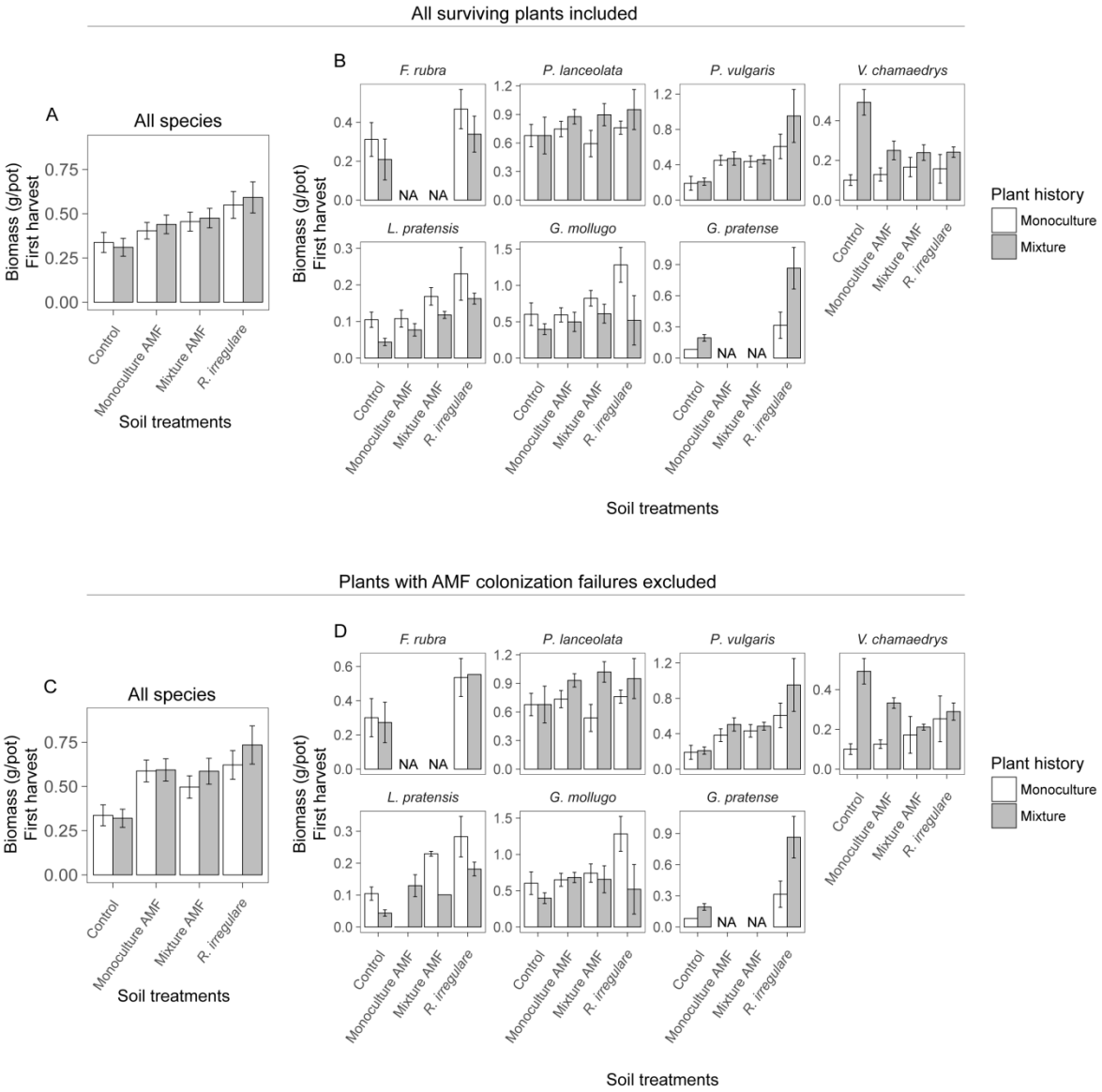

Figure 3. Plant aboveground biomass production of monoculture- (white bars) and mixture-type plants (grey bars) in the four soil treatments at the first harvest: **A**, across all species when all surviving plants are included; **B**, by species when all surviving plants are included; **C**, across all species when plants with AMF colonization failures are excluded (except for soil treatment "Control"); **D**, by species when plants with AMF colonization failures are excluded (except for soil treatment "Control"). Bars represent means ± standard errors.

Table 4. Analysis of variance (ANOVA) for plant biomass at the first harvest.

	All surviving plants included			Plants with AMF colonization failures excluded (except for soil treatment "Control")		
Source of variation	Df	%-SS	*P*	Df	%-SS	*P*
Block	4	6.9	<0.001 ***	4	7.8	<0.001 ***
Functional group (FG)	3	18.0	<0.001 ***	3	12.8	<0.001 ***
Species within FG (SP)	3	20.2	<0.001 ***	3	15.2	<0.001 ***
Plant history (PH)	1	0.1	0.315	1	0.5	0.111
Soil treatment (ST)	3	4.5	<0.001 ***	3	7.8	<0.001 ***
Control vs. AMF treatments (C)	1	2.2	<0.001 ***	1	4.0	<0.001 ***
R. irregulare vs. monoculture or mixture AMF (R)	1	2.2	<0.001 ***	1	3.7	<0.001 ***
Monoculture vs. mixture AMF (F)	1	0.1	0.340	1	0.0	0.750
FG × PH	3	2.1	0.001 **	3	2.6	0.007 **
FG × ST	7	1.5	0.119	7	1.0	0.660
FG × C	3	0.4	0.371	3	0.4	0.562
FG × R	2	0.5	0.121	2	0.4	0.420
FG × F	2	0.5	0.124	2	0.3	0.549
SP × PH	3	1.9	0.002 **	3	2.3	0.013 *
SP × ST	7	2.7	0.005 **	7	2.5	0.104
SP × C	3	1.6	0.006 **	3	1.8	0.040 *
SP × R	2	1.0	0.022 *	2	0.7	0.182
SP × F	2	0.1	0.715	2	0.0	0.898
Residuals	266	33.7		173	36.1	

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

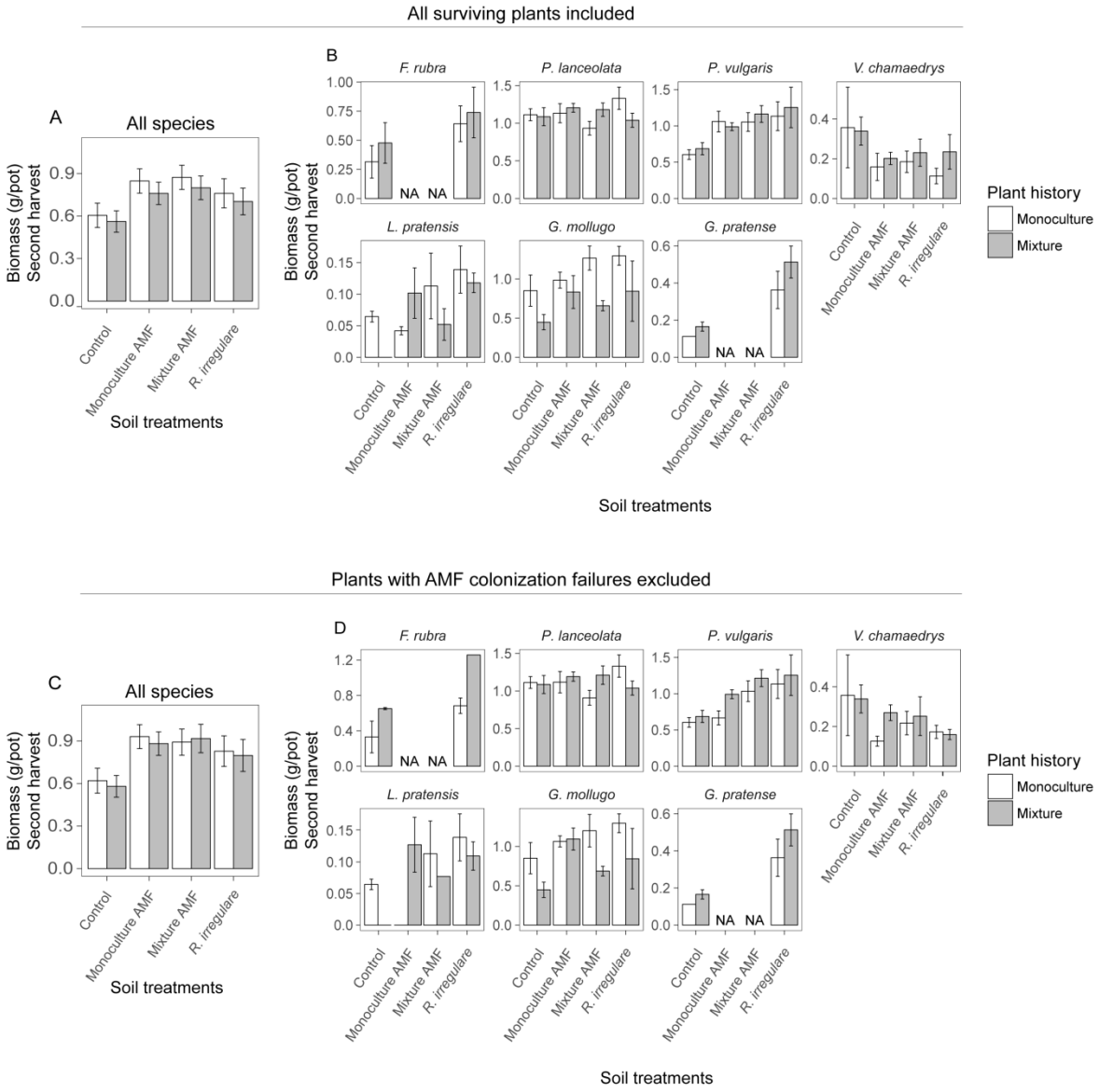

Figure 4. Plant aboveground biomass production of monoculture- (white bars) and mixture-type plants (grey bars) in the four soil treatments at the second harvest: **A**, across all species when all surviving plants are included; **B**, by species when all surviving plants are included; **C**, across all species when plants with AMF colonization failures are excluded (except for soil treatment "Control"); **D**, by species when plants with AMF colonization failures are excluded (except for soil treatment "Control"). Bars represent means ± standard errors.

Table 5. ANOVA for plant biomass at the second harvest.

	All surviving plants included				Plants with AMF colonization failures excluded (except for soil treatment "Control")			
Source of variation	Df	%-SS	*P*		Df	%-SS	*P*	
Block	4	6.8	<0.001	***	4	7.5	<0.001	***
Functional group (FG)	3	16.5	<0.001	***	3	19.6	<0.001	***
Species within FG (SP)	3	41.1	<0.001	***	3	32.2	<0.001	***
Plant history (PH)	1	0.1	0.435		1	0.0	0.794	
Soil treatment (ST)	3	2.8	<0.001	***	3	3.6	<0.001	***
Control vs. AMF treatments (C)	1	2.4	<0.001	***	1	3.0	<0.001	***
R. irregulare vs. monoculture or mixture AMF (R)	1	0.3	0.074	.	1	0.6	0.053	.
Monoculture vs. mixture AMF (F)	1	0.0	0.813		1	0.0	0.738	
FG × PH	3	1.9	0.001	***	3	2.7	0.001	***
FG × ST	7	0.3	0.883		7	0.3	0.972	
FG × C	3	0.1	0.730		3	0.2	0.792	
FG × R	2	0.0	0.954		2	0.1	0.849	
FG × F	2	0.2	0.448		2	0.1	0.831	
SP × PH	3	0.4	0.235		3	0.6	0.229	
SP × ST	7	2.2	0.004	**	7	2.9	0.008	**
SP × C	3	1.9	<0.001	***	3	2.1	0.003	**
SP × R	2	0.1	0.714		2	0.1	0.703	
SP × F	2	0.2	0.316		2	0.7	0.098	.
Residuals	223	22.8			163	23.9		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

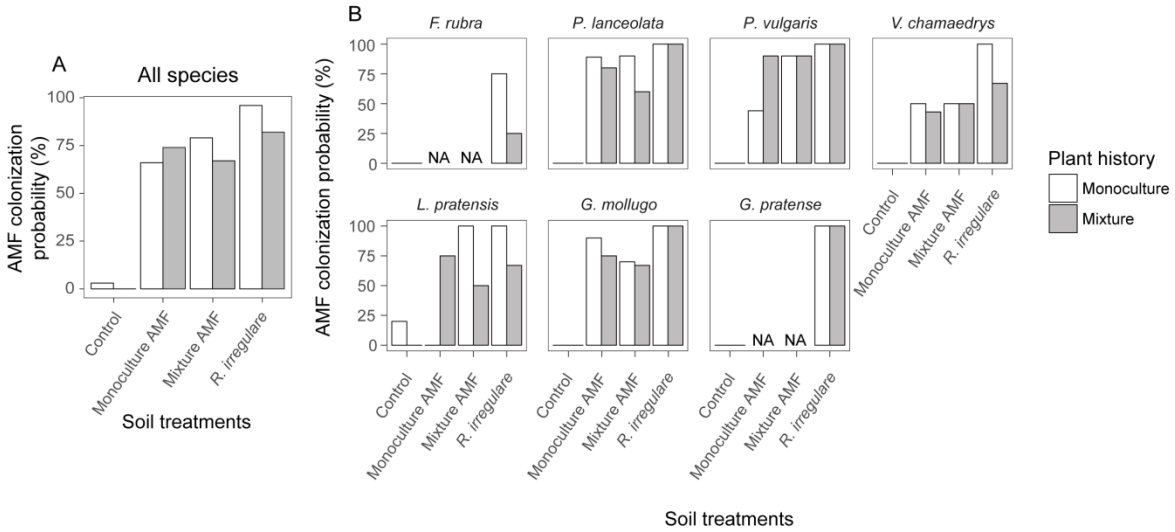

Figure 5. AMF colonization probability in the roots of monoculture- (white bars) and mixture-type plants (grey bars): **A**, across all species; **B**, by species. The bars are proportions of colonized plants out of all plants that survived until the end of the experiment. "NA" indicates that no plants were available of that species for the particular soil treatment.

Table 6. ANDEV for AMF colonization probability of plants.

Source of variation	Df	%-DV	*P*	
Block	4	0.2	0.827	
Functional group (FG)	3	2.4	<0.001	***
Species within FG (SP)	3	2.4	<0.001	***
Plant history (PH)	1	0.1	0.502	
Soil treatment (ST)	3	28.9	<0.001	***
Control vs. AMF treatments (C)	1	26.4	<0.001	***
R. irregulare vs. monoculture or mixture AMF (R)	1	2.5	<0.001	***
Monoculture vs. mixture AMF (F)	1	0.0	0.619	
FG × PH	3	0.4	0.415	
FG × ST	7	0.6	0.705	
FG × C	3	0.0	1.000	
FG × R	2	0.1	0.684	
FG × F	2	0.5	0.129	
SP × PH	3	1.0	0.049	*
SP × ST	7	0.8	0.549	
SP × C	3	0.0	1.000	
SP × R	2	0.2	0.403	
SP × F	2	0.6	0.110	
PH × ST	3	1.1	0.039	*
PH × C	1	0.0	1.000	
PH × R	1	0.7	0.015	*
PH × F	1	0.4	0.086	.
FG × PH × ST	7	1.0	0.395	
FG × PH × C	3	0.0	1.000	
FG × PH × R	2	0.0	1.000	
FG × PH × F	2	1.0	0.022	*
Residuals	223	28.9		

Notes: Df, degrees of freedom; %-DV, proportion of total deviance; *P*, error probability

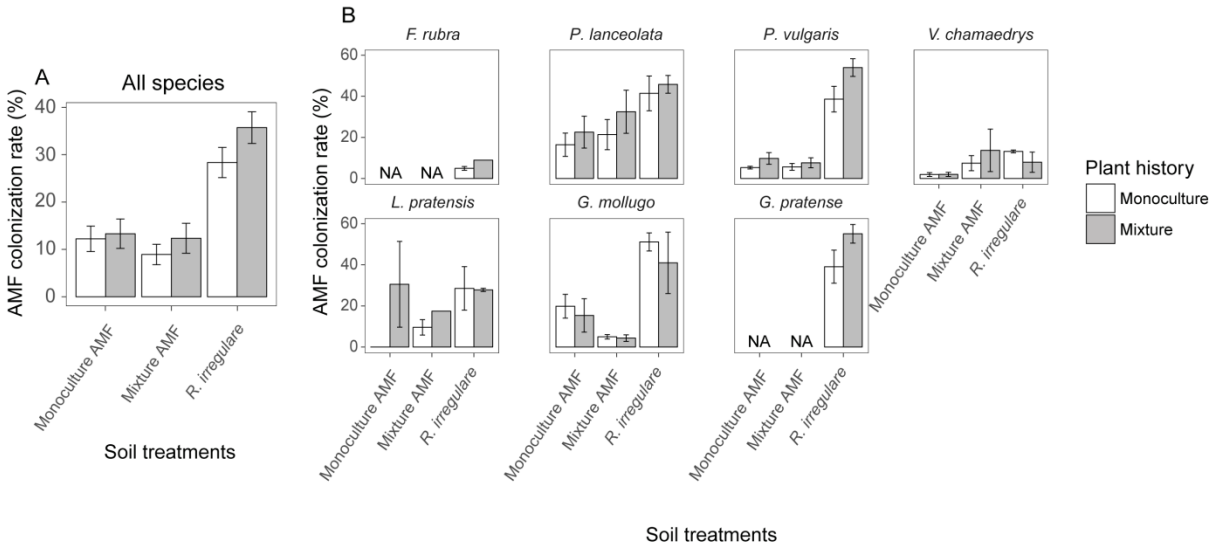

Figure 6. AMF colonization rate in the roots of monoculture- (white bars) and mixture-type plants (grey bars): **A**, across all species; **B**, by species. Only plants with successful AMF-colonization (i.e. colonization rate > 0) were included in calculations and preparation of this figure. Bars are means ± standard errors. "NA" indicates that no plants were available of that species for the particular soil treatment.

Table 7. ANOVA for AMF colonization rate of plants without colonization failure.

Source of variation	Df	%-SS	*P*	
Block	4	2.2	0.061	.
Functional group (FG)	3	2.6	0.016	*
Species within FG (SP)	3	12.0	<0.001	***
Plant history (PH)	1	0.8	0.065	.
Soil treatment (ST)	2	19.6	<0.001	***
R. irregulare vs. monoculture or mixture AMF (R)	1	19.4	<0.001	***
Monoculture vs. mixture AMF (F)	1	0.1	0.468	
FG × PH	3	0.4	0.684	
FG × ST	4	3.2	0.012	*
FG × R	2	1.2	0.086	.
FG × F	2	2.0	0.018	*
SP × PH	3	0.5	0.593	
SP × ST	4	3.5	0.008	**
SP × R	2	3.0	0.003	**
SP × F	2	0.5	0.376	
PH × ST	2	0.0	0.977	
PH × R	1	0.0	0.926	
PH × F	1	0.0	0.846	
Residuals	122	29.1		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

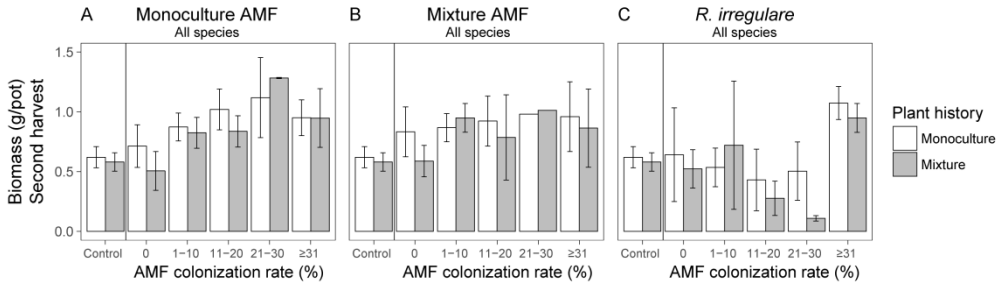

Figure 7. Aboveground biomass production of monoculture- (white bars) and mixture-type plants (grey bars) in control soil and under different AMF colonization rates of: **A**, Monoculture AMF across all species; **B**, Mixture AMF across all species; **C**, *R. irregulare* across all species. Bars are means ± standard errors.

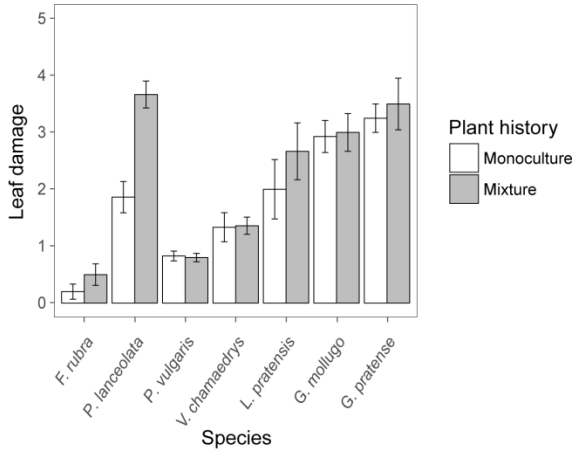

Figure 8. Amount of leaf damage (estimated from no damage (0) to strong damage (5)) of monoculture-type (white bars) and mixture-type plants (grey bars). Bars represent means ± standard errors.

Table 8. ANOVA for plant leaf damage.

	All surviving plants included				Plants with AMF colonization failures excluded (except for soil treatment "Control")			
Source of variation	Df	%-SS	*P*		Df	%-SS	*P*	
Block	4	5.1	<0.001	***	4	4.5	0.001	***
Functional group (FG)	3	21.6	<0.001	***	3	23.3	<0.001	***
Species within FG (SP)	3	19.2	<0.001	***	3	19.1	<0.001	***
Plant history (PH)	1	2.3	0.001	***	1	3.4	<0.001	***
Soil treatment (ST)	3	0.1	0.874		3	0.0	0.997	
Control vs. AMF treatments (C)	1	0.0	0.705		1	0.0	0.840	
R. irregulare vs. monoculture or mixture AMF (R)	1	0.1	0.497		1	0.0	0.938	
Monoculture vs. mixture AMF (F)	1	0.0	0.763		1	0.0	0.997	
FG × PH	3	0.6	0.389		3	1.1	0.180	
FG × ST	7	1.3	0.458		7	3.3	0.043	*
FG × C	3	0.7	0.275		3	0.6	0.435	
FG × R	2	0.3	0.439		2	1.2	0.070	.
FG × F	2	0.2	0.549		2	1.5	0.037	*
SP × PH	3	5.0	<0.001	***	3	5.1	<0.001	***
Residuals	230	43.4			170	37.1		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

SUPPORTING INFORMATION

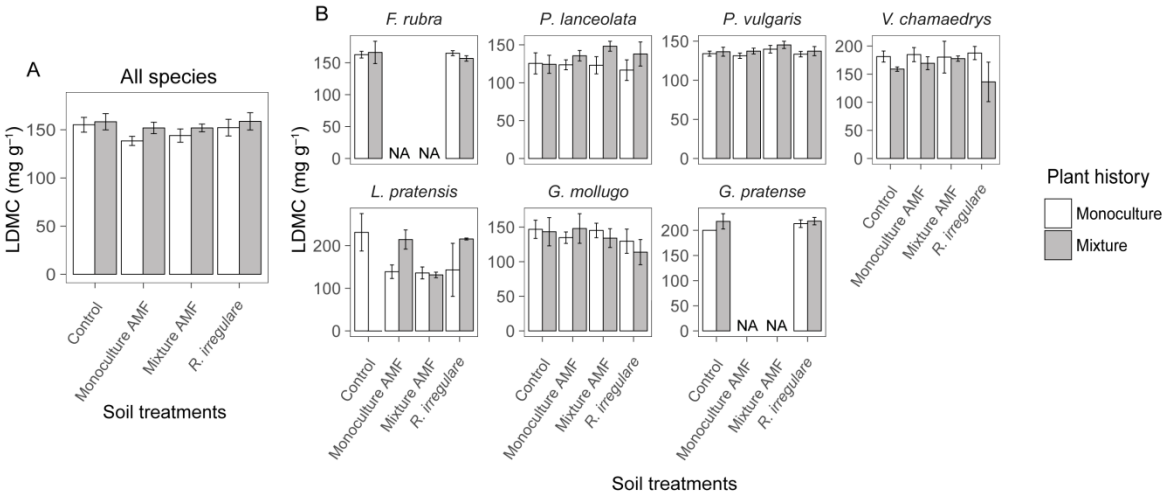

Figure S1. LDMC of monoculture- (white bars) and mixture-type plants (grey bars) in the four soil treatments: **A**, across all species when all surviving plants are included; **B**, by species when all surviving plants are included. Bars represent means ± standard errors. "NA" indicates that no plants were available of that species for the particular soil treatment.

Table S1. ANOVA for plant LDMC.

Source of variation	Df	%-SS	*P*	
Block	4	2.5	0.024	*
Functional group (FG)	3	5.5	<0.001	***
Species within FG (SP)	3	27.3	<0.001	***
Plant history (PH)	1	0.2	0.346	
Soil treatment (ST)	3	0.4	0.574	
Control vs. AMF treatments (C)	1	0.0	0.732	
R. irregulare vs. monoculture or mixture AMF (R)	1	0.4	0.210	
Monoculture vs. mixture AMF (F)	1	0.1	0.588	
FG × PH	3	0.9	0.241	
FG × ST	7	5.3	0.002	**
FG × C	3	3.1	0.004	**
FG × R	2	0.8	0.182	
FG × F	2	1.4	0.041	*
SP × PH	3	1.9	0.037	*
Residuals	227	50.3		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

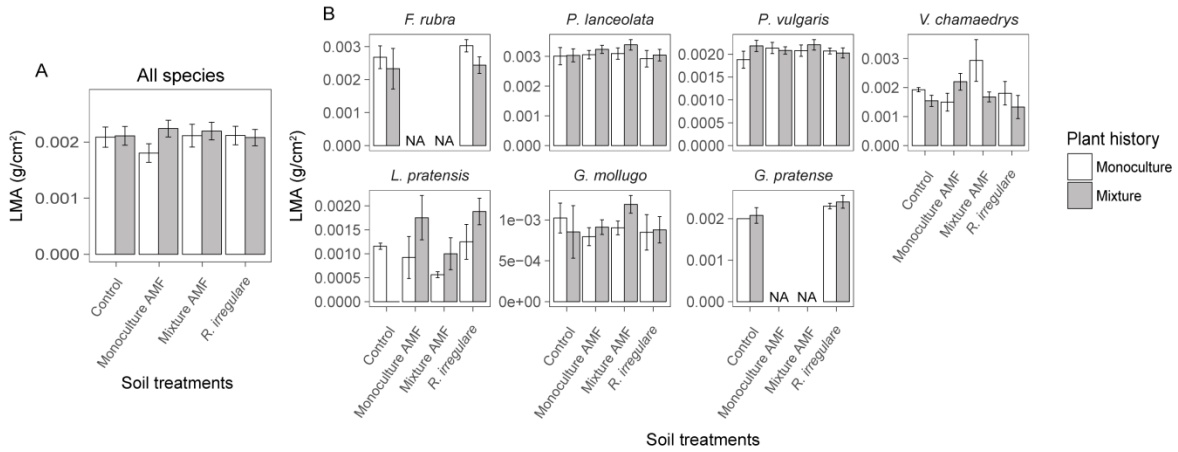

Figure S2. LMA of monoculture- (white bars) and mixture-type plants (grey bars) in the four soil treatments: **A**, across all species when all surviving plants are included; **B**, by species when all surviving plants are included. Bars represent means ± standard errors. "NA" indicates that no plants were available of that species for the particular soil treatment.

Table S2. ANOVA for plant LMA.

Source of variation	Df	%-SS	*P*	
Block	4	3.8	<0.001	***
Functional group (FG)	3	30.4	<0.001	***
Species within FG (SP)	3	26.0	<0.001	***
Plant history (PH)	1	0.0	0.920	
Soil treatment (ST)	3	0.6	0.201	
Control vs. AMF treatments (C)	1	0.3	0.149	
R. irregulare vs. monoculture or mixture AMF (R)	1	0.2	0.244	
Monoculture vs. mixture AMF (F)	1	0.2	0.273	
FG × PH	3	0.9	0.081	.
FG × ST	7	1.4	0.150	
FG × C	3	0.1	0.777	
FG × R	2	0.8	0.045	*
FG × F	2	0.5	0.175	
SP × PH	3	1.1	0.041	*
SP × ST	7	0.7	0.581	
SP × C	3	0.2	0.629	
SP × R	2	0.4	0.237	
SP × F	2	0.1	0.600	
PH × ST	3	0.4	0.441	
PH × C	1	0.0	0.954	
PH × R	1	0.0	0.653	
PH × F	1	0.3	0.115	
FG × PH × ST	6	0.3	0.879	
FG × PH × C	2	0.1	0.695	
FG × PH × R	2	0.0	0.896	
FG × PH × F	2	0.2	0.488	
SP × PH × ST	7	2.6	0.007	**
SP × PH × C	3	0.1	0.848	
SP × PH × R	2	0.0	0.988	
SP × PH × F	2	2.4	<0.001	***
Residuals	204	26.1		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

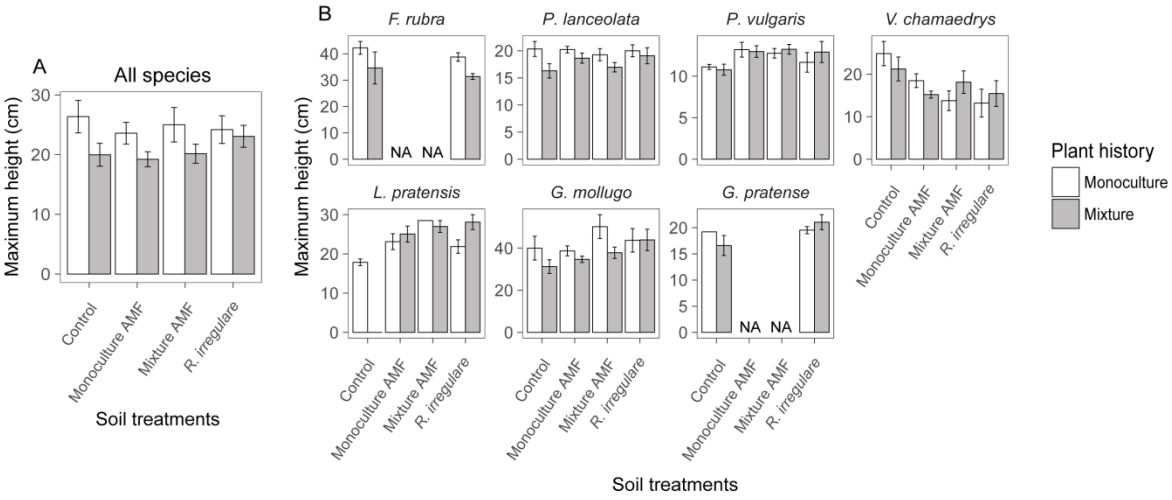

Figure S3. Maximum height of monoculture- (white bars) and mixture-type plants (grey bars) in the four soil treatments: **A**, across all species when all surviving plants are included; **B**, by species when all surviving plants are included. Bars represent means ± standard errors. "NA" indicates that no plants were available of that species for the particular soil treatment.

Table S3. ANOVA for maximum plant height.

Source of variation	Df	%-SS	*P*	
Block	4	0.8	0.065	.
Functional group (FG)	3	54.9	<0.001	***
Species within FG (SP)	3	16.7	<0.001	***
Plant history (PH)	1	0.5	0.020	*
Soil treatment (ST)	3	0.3	0.323	
Control vs. AMF treatments (C)	1	0.1	0.345	
R. irregulare vs. monoculture or mixture AMF (R)	1	0.0	0.661	
Monoculture vs. mixture AMF (F)	1	0.2	0.122	
FG × PH	3	1.6	0.001	***
FG × ST	7	2.1	0.001	**
FG × C	3	0.7	0.043	*
FG × R	2	0.1	0.692	
FG × F	2	1.3	0.001	***
SP × PH	3	0.6	0.071	.
Residuals	230	20.1		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

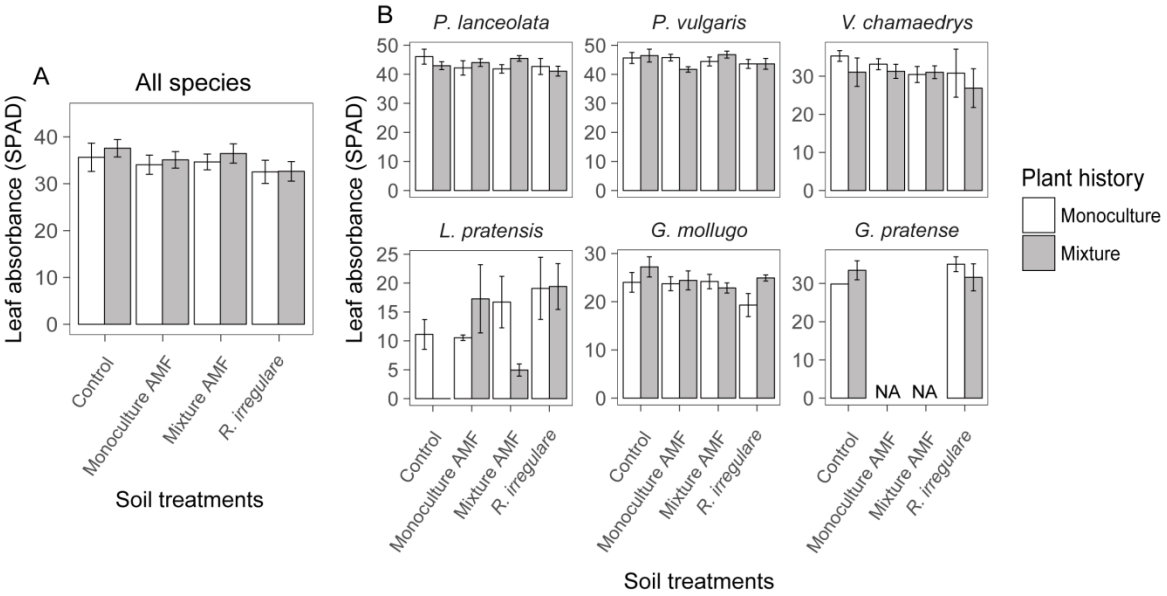

Figure S4. Leaf absorbance (SPAD) of monoculture- (white bars) and mixture-type plants (grey bars) in the four soil treatments: **A**, across all species when all surviving plants are included; **B**, by species when all surviving plants are included. Bars represent means ± standard errors. "NA" indicates that no plants were available of that species for the particular soil treatment.

Table S4. ANOVA for plant leaf absorbance.

Source of variation	Df	%-SS	*P*	
Block	4	3.2	<0.001	***
Functional group (FG)	2	54.0	<0.001	***
Species within FG (SP)	3	20.2	<0.001	***
Plant history (PH)	1	0.0	0.983	
Soil treatment (ST)	3	0.2	0.547	
Control vs. AMF treatments (C)	1	0.1	0.196	
R. irregulare vs. monoculture or mixture AMF (R)	1	0.0	0.525	
Monoculture vs. mixture AMF (F)	1	0.0	0.831	
FG × PH	2	0.1	0.751	
FG × ST	6	1.2	0.031	*
FG × C	2	0.3	0.203	
FG × R	2	0.7	0.015	*
FG × F	2	0.2	0.298	
SP × PH	3	0.4	0.224	
SP × ST	7	0.2	0.900	
SP × C	3	0.1	0.753	
SP × R	2	0.0	0.881	
SP × F	2	0.1	0.507	
PH × ST	3	0.0	0.935	
PH × C	1	0.0	0.966	
PH × R	1	0.0	0.656	
PH × F	1	0.0	0.638	
FG × PH × ST	5	1.3	0.008	**
FG × PH × C	1	0.1	0.270	
FG × PH × R	2	0.2	0.385	
FG × PH × F	2	1.1	0.002	**
Residuals	200	16.4		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

Chapter 3:
Co-selection of plants and soil organisms in a biodiversity experiment alters plant phenotype, microbial composition and plant–microbe interactions

Co-selection of plants and soil organisms in a biodiversity experiment alters plant phenotype, microbial composition and plant–microbe interactions

This chapter will be divided into several paper manuscripts. Author order has not been finalized.

Authorship

Terhi Hahl[1], Cameron Wagg[1], Sofia J. van Moorsel[1], Gerlinde de Deyn[2] and Bernhard Schmid[1] conceived the study; Terhi Hahl carried out the experiment, collected data and prepared the metagenomic sequencing library; Terhi Hahl and Bernhard Schmid analysed the plant data and wrote the manuscript; Marc W. Schmid[1] analysed the sequencing data and wrote the corresponding sections in Methods and Results; Yvonne Forster[3] and Laurent Bigler[3] measured and analysed plant leaf chemistry and wrote the corresponding section in Methods.

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ABSTRACT

While increasing evidence suggests that soil microbes play a crucial role in the maintenance of plant diversity and productivity, it is unclear how the loss of plant diversity influences the phenotypes of plants, the biodiversity of soil microbes and plant–microbe interactions in the longer term. We hypothesize that co-selection of plants and soil microbes over 11 years in plant monocultures and mixtures of a biodiversity experiment leads to differences in plant phenotypes and microbial composition. Additionally, we hypothesize that soil microbiomes of monocultures and mixtures alter plant performance differentially in "home" and "away" combinations. We tested our hypotheses by growing plants selected in monocultures (monoculture-type plants) and species mixtures (mixture-type plants) individually in the presence and absence of full or simplified communities of co-selected soil organisms. We show that selection in monocultures vs. mixtures alters the phenotypes of plants and the biodiversity of soil microbes. We furthermore show that selection in monocultures leads to plant genotype-specific negative effects of soil microbiota in "home" combinations but not in "away" combinations. Finally, we show that higher soil biodiversity protects plants from these negative effects.

Key words*:* arbuscular mycorrhizal fungi, rhizosphere bacteria, pathogen accumulation, plant diversity, selection, soil biodiversity

INTRODUCTION

Plants as primary producers and connectors of below- and aboveground biodiversities (van Dam & Heil, 2011) have a vital role in ecosystem functioning and ecosystem services (Tilman & Downing, 1994; Naeem *et al.*, 1994; Daily, 1997) but the global decline of plant biodiversity is feared to decrease the functioning of ecosystems and the services they provide (Cardinale *et al.*, 2012). When it comes to the concerns about plant biodiversity, the consideration of soil biodiversity is equally important: increasing evidence suggests that soil microbes play a crucial role in the maintenance of plant biodiversity (Petermann *et al.*, 2008; Bever *et al.*, 2015). However, the long-term influence of biodiversity loss on the specific interactions between plants and soil organisms and the further consequences on plant productivity are not well understood, in particular because the interactions may change over time.

Plants live in association with a remarkable amount of microbial soil organisms (Bulgarelli *et al.*, 2013). These interactions take place in the rhizosphere, a narrow zone of soil surrounding plant roots (Whipps, 2001). The composition of microbes in the rhizosphere is influenced by the local biotic and abiotic conditions (van der Putten *et al*., 2013). Plants may influence these conditions by the secretion of chemical substances, root morphology, inputs of organic matter, changes in soil moisture, pH and surface soil temperature, and by providing habitats and resources to various soil organisms (Bardgett & Wardle, 2003; Berg & Smalla, 2009; van Dam, 2009; Bulgarelli *et al.*, 2013; Latz *et al.*, 2016). Thereby, plants can initiate large changes in the microbial composition of the rhizosphere (Dakora & Phillips, 2002; Latz *et al.*, 2016). Because the conditions in the rhizosphere tend to vary between plant species (Berg & Smalla, 2009; Latz *et al.*, 2016; Eisenhauer *et al.*, 2017), loss of plant species diversity has the potential to fuel biodiversity loss in soil (Hooper *et al.*, 2000; Broughton & Gross, 2000; Garbeva *et al.*, 2006; Schlatter *et al.*, 2015). Soil microbes play an essential role in a number of ecosystem functions including decomposition, nutrient cycling, nutrient retention, plant biomass production, plant nutrient uptake and the maintenance of plant species diversity (Ahemad & Kibret, 2014; Wagg *et al*., 2014; Bever *et al*., 2015). Loss of soil biodiversity can endanger the optimal functioning of ecosystems by breaking up networks of organisms and disturb the processes performed by soil microbes (Gosling *et al.*, 2006; Wagg *et al.*, 2014). In addition, loss of soil biodiversity can expose plants to specialized enemies from which they are normally protected by the large biodiversity of other soil organisms (Eisenhauer *et al.*, 2012; van der Putten *et al.*, 2013; Ahemad & Kibret, 2014).

While plants may alter microbial composition in the rhizosphere soil, the composition of soil microbes can, in turn, drive changes in the plant community composition by altering the growth and survival of individual plants (Bever *et al.*, 2015). The influence of soil microbes on plant survival and growth can vary between positive and negative. The net effects are called positive and negative plant–soil feedbacks, respectively (van der Putten *et al.*, 2013). Among the soil microbiota that plants interact with, positive feedbacks are typically promoted by plant-beneficial arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) (van der Heijden *et al.*, 2008; Ahemad & Kibret, 2014). AMF are ubiquitous fungi from the division of Glomeromycota, known to form symbiotic associations with a majority of land plants (van der Heijden *et al.*, 2008). In

exchange of the carbon provided by the host plant, AMF can promote plant growth and survival, and protect plants from various stressors by increasing nutrient and water uptake of the plant (van der Heijden *et al.*, 1998; Augé, 2001; Smith & Smith, 2011). In addition, AMF have been shown to promote plant tolerance and defense mechanisms against pests (Newsham *et al.*, 1995; Rodriguez & Redman, 2008; Tao *et al.*, 2016). The other group of beneficial soil organisms, the PGPR, represent 2–5 % of bacteria found in the rhizosphere, and can promote plant growth directly or indirectly (Ahemad & Kibret, 2014). Direct mechanisms include improved acquisition of nutrients such as nitrogen and phosphorus and alterations of plant physiological processes (Lugtenberg & Kamilova, 2009; Glick, 2012; Ahemad & Kibret, 2014). Indirectly, PGPR may promote plant growth by competing against pathogenic microbes in the available niche space and resources, by producing antifungal metabolites and by inducing plant defense responses (Lugtenberg & Kamilova, 2009; Glick, 2012; Ahemad & Kibret, 2014).

Soil pathogens, on the other hand, are typically responsible for negative feedbacks on plants (van der Putten *et al.*, 2013). Soil pathogens represent a wide group of organisms, including bacteria, fungi, viruses and water molds (reviewed by Bever *et al.*, 2015). Common to all of them is their negative effects on plant survival and growth occurring for instance as seed decays, increased seedling mortality or root necrosis (Bever *et al.*, 2015). Plant pathogens are often classified as generalists (many host plant species) and specialists (one or few host plant species) according to the range of plant species they consume (Barrett and Heil 2012). Among various root-associated microbes that plants interact with, specialized pathogens play a particularly important role in the structuring of plant communities (Petermann *et al.*, 2008; Schnitzer *et al.*, 2011; Maron *et al.*, 2011; Bever *et al.*, 2015). Species-specific soil pathogens tend to accumulate nearby the dominant plant species of the community and consequently reduce the growth of the species (Petermann *et al.*, 2008). In monocultures, accumulating specialized pathogens have particularly strong negative effects on plant growth. In diverse plant communities, however, the negative effects of specialized pathogens normally dilute (Petermann *et al.*, 2008; Eisenhauer *et al.*, 2012; van der Putten *et al.*, 2013).

Diversity-dependent accumulation of specialized plant-enemies may take place not only below but also above ground (Janzen, 1970; Burdon & Chilvers, 1982; Petermann *et al.*, 2008). Plants may avoid negative effects of such below- and aboveground enemies by an association with beneficial soil organisms but also by allocating resources for defense rather than for growth (Herms & Mattson, 1992). Direct defenses that plants use against their enemies can be roughly divided into toxins and morphological traits (van der Putten *et al.*, 2001; Agrawal, 2007). Toxins are part of a large group of secondary metabolites that plants produce against pathogens and herbivores but also for many other functions such as attraction of pollinators and protection from osmotic stress (Bennett & Wallsgrove, 1994; Bourgaud *et al.*, 2001; Wallace, 2004). Many defensive secondary metabolites are targeted specifically towards herbivores or pathogens but also general defense compounds have been found (Biere *et al.*, 2004). In addition, defensive secondary metabolites are linked between below- and aboveground in a way that belowground enemies can also cause defense responses to aboveground enemies and aboveground enemies to belowground enemies (Bezemer & van Dam, 2005). Defensive morphological traits of plants, on the other hand, include special

physically protective structures such as trichomes (Agrawal, 1999), increased toughness or hardness of leaves (Coley, 1988; Turner, 1994) and roots (van Dam, 2009). Their defensive properties are mainly based on deterrence or reduced digestibility of plant tissues (Turner, 1994; Agrawal, 1999).

Long-term consequences of plant biodiversity loss in soil biodiversity and the further feedbacks on plant productivity have become possible to study in controlled conditions with long-running biodiversity experiments (Tilman *et al.*, 2006; Eisenhauer *et al.*, 2011; Roscher *et al.*, 2013; Zuppinger-Dingley *et al.*, 2016). Additionally, such experiments, of which many were planted in 1990s and early 2000s (reviewed in Cardinale *et al.*, 2012; Eisenhauer *et al.*, 2016), have shown a potential to be used as selection experiments: local selection pressures in the test monocultures and mixtures have been found to favour different plant traits and lead to a formation of low- and mid-diversity adapted pools of plant genotypes (Zuppinger-Dingley *et al.*, 2014, 2015, 2016). Here we refer to the monoculture- and mixture-selected plant genotypes as monoculture- and mixture-type plants, respectively. The formation of such plant types is assumed to take place through a sorting-out from an initially larger standing variation of co-existing individuals (Zuppinger-Dingley *et al.*, 2014). Individuals with traits that fit relatively better to the local conditions of a specific community diversity have higher chances to survive and thereby increase the genetic representation in the community (Bossdorf *et al.*, 2008). Because the process changes allele frequencies in the local populations, and thereby fulfils the definition of evolution, the formation of such selected pools of genotypes can be referred to as rapid evolution (Hairston *et al.*, 2005).

In previous studies, monoculture- and mixture-type plants were distinguishable from each other after eight years of selection based on differences in combining ability (Zuppinger-Dingley *et al.*, 2014) and foliar metabolic fingerprints (Zuppinger-Dingley *et al.*, 2015). In addition, after eight years of co-selection of plants and soil communities, Zuppinger-Dingley *et al.* (2016) found in their experiment that the feedbacks of monoculture soil were positive for monoculture-type plants but negative for mixture-type plants. The authors suggested that an accumulation of specialized pathogens in monocultures, and their dilution in mixtures, could create differential selection pressure depending on the local plant species diversity. The finding led to a hypothesis suggesting that the accumulation of species-specific pathogens in monocultures over time could favour plants that trade off growth for better defense against accumulating specialized pathogens (Zuppinger-Dingley *et al.*, 2016). The authors also proposed an alternative hypothesis, suggesting that the pathogen pressure could also favour plants that are better protected from the enemies by a stronger association with beneficial soil microbes. These theories, however, remain to be tested in practice.

In the present study, we examined the interactions of plants selected for over 11 years in monocultures (monoculture-type plants) and species mixtures (mixture-type plants), and soil communities co-selected with the studied plants for eight plus three years (a mixing of soils took place after the first eight years of selection). To disentangle the specific influence of different groups of soil organisms on plant phenotypes, we measured biomass production and a number of other traits of plants grown in a full soil community (field-soil treatment), in a simplified soil community without organisms larger than 25 µm in diameter (microbial soil treatments) or in sterilized soil without living organisms (control-soil treatment). Additionally, we tested the influence of plant species identity and diversity on the

composition of soil microbiota. Our aim was to test whether co-selection of plants and soil organisms over 11 years in monocultures and mixtures in a biodiversity experiment (the Jena Experiment) alters plant phenotypes and soil microbial composition. Based on the hypothesis suggested by Zuppinger-Dingley et al. (2016) we expected that, in response to a potential accumulation of specialized pathogens in monocultures (Petermann *et al.*, 2008), selection in monocultures favours plants that trade off biomass production for improved defense. As an alternative hypothesis, we anticipated that selection in monocultures favours plants that associate stronger with beneficial soil microbes. In terms of soil microbes, we hypothesized that different plant species and plant species diversities assemble distinct microbiomes in the rhizosphere soils. Finally, we tested whether co-adaptation of plants and soil microbes leads to increased or decreased plant performance in "home"-combinations (monoculture-type plants with monoculture microbes and mixture-type plants with mixture microbes) as compared with "away"- combinations (monoculture-type plants with mixture microbes and mixture-type plants with monoculture microbes).

METHODS

The present study included eight common European grassland plant species from four different functional groups: one grass (*Festuca rubra* L.), three small herbs (*Plantago lanceolata* L., *Prunella vulgaris* L., and *Veronica chamaedrys* L.), two tall herbs (*Galium mollugo* L. and *Geranium pratense* L.) and two legumes (*Lathyrus pratensis* L. and *Onobrychis viciifolia* Skop.). The studied species had undergone 11 years of selection in either plant monocultures (monoculture-type plants) or species mixtures (mixture-type plants) from 2002–2014 (Fig. 1, upper part).

First controlled seed production and "soil training"

The plant communities of 48 plots (12 monocultures, 12 2-species mixtures, 12 4-species mixtures and 12 8-species mixtures) of a biodiversity experiment in Jena, Germany, the Jena Experiment (Roscher *et al.*, 2004), were collected as cuttings in spring 2010, after eight years of selection in their respective plant communities, and transplanted in identical plant composition to an experimental garden in Zurich, Switzerland, for the first controlled sexual reproduction among co-selected plants (for details see (Zuppinger-Dingley *et al.*, 2014). In addition, the top 30 cm of soil of the 48 plots was pooled together, mixed and placed back to the excavated locations in the Jena Experiment. In spring 2011, the seedlings produced from the seeds of the first controlled sexual reproduction were transplanted back to the mixed soil in the same plots of the Jena Experiment from where the parents had originally been collected and in the same community composition as the parents had been established. These plant communities were maintained for another three years until 2014 to allow them to become associated again with their own microbial communities and continue the selection treatments in their respective communities (Fig. 1, upper part).

Second controlled seed production

The seeds used in the present study were obtained from a second controlled seed production. In March 2014, the plant communities of the re-established plots in the Jena Experiment were brought back to the plots of the experimental garden in Zurich. The plots had been filled with 30 cm of soil (1:1 mixture of garden compost and field soil, pH 7.4, Gartenhumus, RICOTER Erdaufbereitung AG, Aarberg, Switzerland) and fenced with netting to minimize cross-pollination with plants outside the plots. Seeds of eight monoculture plots, one four-species mixture plot and six eight-species mixture plots of 1x1 m in the experimental garden were collected for the present experiment (see Table S1 for the species composition of the mixture plots). After collection, the seeds of the eight plant species were stored at +4 ˚C for at least two months. Two to four weeks before the start of the plant–soil feedback experiment reported in this study, depending on pre-tested germination times of each species, we surface-sterilized the seeds with bleach under constant stirring using pre-tested bleach concentrations and sterilization times (*L. pratensis* and *O. viciifolia*: 14 % bleach for 40 min; *G. mollugo*: 14 % bleach for 20 min; *G. pratense*: 7 % bleach for 10 min, all other species: 7 % bleach for 5 min), rinsed the seeds 10 times in autoclaved water and germinated them on 1 % water-agar.

Soil collection and inoculum preparation

In March 2014, rhizosphere soil samples attached to the roots of the plants that we transported to Zurich for the second sexual reproduction were collected and stored at 4 °C. By the time of our soil sampling, the soil communities had thus undergone eight plus three years of community assembly and potential co-evolution with each of the eight plant species in monocultures (monoculture-type plants) or mixtures (mixture-type plants).

To isolate microbial communities but exclude AMF spores from the sampled rhizosphere soils, we passed 500 ml of deionized water and 25 g of rhizosphere soil samples through a series of sieves with the smallest mesh size of 25 µm (Wagg *et al.*, 2014) — AMF spores are larger than 25 µm in diameter; Fig. 1, fourth and fifth row from bottom). To allow accumulation of the isolated microbes, we established trap cultures that consisted of 2 L of 4:1 sand-soil mixture, autoclaved at 120 °C for 99 min, and monocultures of trap plants of each of the eight tested plant species (Fig. 1, third row from bottom). The 500 ml of microbial wash was divided between two trap culture replicates. Thus, all trap cultures received 250 ml of microbial wash. For the trap plants, we used seeds from a commercial seed supplier who had also provided the original seed material for the Jena Experiment (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany) to avoid that the trap plants and microbes collected from the rhizosphere of monoculture- and mixture-type plants shared a common selection history. Before planting to trap cultures, these seeds were surface-sterilized and pre-germinated on 1 % water-agar. To avoid cross-contamination between trap-cultures, each pot was kept on a saucer and watered individually from top by avoiding water splashing. All pots and saucers were autoclaved before the experiment at 120 °C for 20 min.

After five months of growth in the glasshouse, we collected a root sample from each trap culture, fixed the root samples in 50 % ethanol, cleared them with 10 % KOH, stained them with 5 % ink-vinegar (Vierheilig *et al.*, 1998), and confirmed the absence of AMF colonization microscopically. The trap plants were harvested at ground level, the belowground content of the replicated trap cultures pooled, the roots cut into 3–5 cm

fragments, and the belowground content of the trap cultures used as soil inoculum in the plant–soil feedback experiment described below.

To prepare an additional inoculum of field soil unique to each plant species and plant history, we collected soil in January 2015 from the same eight monoculture plots and seven mixture plots of the experimental garden from which the seeds of the particular plant species and plant history of the present experiment were collected and mixed 1:1 with the stored rhizosphere soil samples collected from the corresponding plant species and plant histories.

Setup of plant–soil feedback experiment

To standardize nutrient composition of the soil treatments of the present study, we used soil inoculum from three sources, monoculture and mixture microbes from trap cultures and field soil. One of the sources was live inoculum and two autoclaved for 99 min at 120 °C, except for the control-soil treatment which received autoclaved inoculum from all three sources. We filled 1-L pots with 5.6 dl of gamma-radiated (27–53 kGy) 1:1 (w/w) sand-soil mixture, added 0.8 dl inoculum from the three sources, and covered the inoculum with 1 dl of the gamma-radiated sand-soil mixture to avoid cross-contamination of the live soil inoculum between pots. The soil treatments thus were a) control (no live inoculum), b) monoculture microbes (9 % (v/v) live inoculum of monoculture microbe trap culture substrate), c) mixture microbes (9 % live inoculum of mixture microbe trap culture substrate and d) field soil (9 % live inoculum of home field soil of the corresponding plant species and plant history). One pre-germinated monoculture- or mixture-type plant seedling of one of the eight test species was planted to each pot. The experiment included in total eight species, four soil treatments and two plant histories (monoculture- and mixture-type plants). The setup of the experiment was full factorial for the eight species and two plant histories grown in the soil treatments of control, monoculture microbes and mixture microbes. The field-soil treatment, instead, was unique to each plant species and plant history. Each treatment combination was replicated seven times resulting in 448 pots that were randomly arranged within seven experimental bocks in a glasshouse compartment, ensuring that each treatment combination occurred only once in each block.

To avoid contamination of the soil during the setup of the experiment, all pots and saucers were autoclaved before the experiment for 20 min at 120 °C and each soil inoculum was handled using pre-sterilized tools dedicated to the specific inoculum. Cross-contamination of the soil treatments was further avoided by cleaning hands and tools in 70 % ethanol between working steps. Each pot was kept on a saucer and individually watered every second or third day from top by avoiding water splashes between pots.

Plant mortality due to larvae of fungus gnat (*Bradysia* spp.)

Some plant mortality during the experiment was caused by fungus gnats *(Bradysia spp.).*

Aboveground leaf damage due to common glasshouse pests

We found powdery mildew (family Erysiphaceae) infections on *P. lanceolata* and *G. mollugo,* two-spotted spider mite (*Tetranychus urticae* Koch) damage on *G. pratense, L. pratensis* and *O. viciifolia,* and white fly (family Aleyrodidae) damage mainly on *G. pratense*

and *P. vulgaris*. In addition, we observed signs of pest damage on the leaves of *F. rubra* and *V. chamaedrys* but could not specify which organism caused the damage.

Data collection

We harvested plant aboveground biomass at 4 cm above soil level after 11–15 weeks of plant growth (first harvest, Table 2). Eight weeks after the first harvest, we harvested the biomass at soil level (second harvest, Table 2). The biomass of each plant was dried at 70 °C for 48 h and weighed. At the second harvest, we additionally measured plant maximum height and mean leaf absorbance (SPAD-502Plus Chlorophyll Meter, KONICA MINOLTA, INC., Osaka, Japan) of three representative leaves of each plant and estimated the degree of pest damage at plant above-ground tissues (Table 2). We assessed leaf mass per area (LMA) and leaf dry matter content (LDMC) by measuring the area (LI-3100C Area Meter, LI-COR, Lincoln, USA) of fresh representative leaves immediately after harvest and weighing the leaves before and after drying at 70 °C for 48 h (Table 2). Leaf absorbance of *F. rubra* was not measured because of too narrow leaves. For the analysis of leaf chemistry, we collected a subsample of leaves of each plant. These leaf subsamples were shock-frozen in liquid nitrogen, freeze-dried for 72 hours to ambient dry-mass, weighed and stored in sealed plastic bags with silica gel at room temperature until further processing. To assess the total biomass of each plant at the second harvest (Bm2), we summed the dry biomass of leaf samples used for the LMA and LDMC measurements, leaf samples that were freeze-dried and the remaining above-ground biomass of the plant.

To determine the AMF colonization of plant roots at the end of the experiment, a random subsample of roots were washed, cut into small fragments and stored in 50 % ethanol until root clearing, staining and microscopy using the method described above (Vierheilig *et al.*, 1998). After the root-washing step, we additionally estimated the degree of root damage based on the proportion of dark-coloured roots due to root necrosis (Table 2).

For the analysis of soil microbiota, we sampled a random subset of rhizosphere of each plant immediately after the harvest and stored at –80 °C until DNA extraction. To avoid cross-contamination of the soil samples, all material was cleaned in 70 % ethanol between working steps.

Analysis of plant survival and traits

The plant biomass response to live soil in comparison with the control-soil treatment was calculated by subtracting plant biomass produced in live soil treatment from plant biomass produced in the corresponding control-soil treatment and dividing the value by the mean of the two. Biomass response of plants at the two harvests, plant trait measurements, leaf and root damage estimates and AMF colonization rate in home soil were analysed using linear models and analysis of variance (ANOVA). Early (plants that died before the first harvest) and late plant survival (plants that died after the first harvest but before the second harvest) were analysed using logistic models and analysis of deviance. The results were summarized in ANOVA and ANDEV tables, respectively (McCullagh & Nelder, 1998; Schmid *et al.*, 2017). The explanatory terms of the models were experimental block, table, plant functional group, species within functional group, soil treatments (all soil treatments as groups of a four-level factor or the following orthogonal contrast terms: control vs. live soil treatments, field

soil vs. monoculture or mixture microbes and monoculture vs. mixture microbes), plant history (monoculture- vs. mixture-type plants) and interactions of these. Of particular interest was the interaction of the soil-treatment contrast monoculture vs. mixture microbes with plant history, because this corresponded to a comparison between "home" (monoculture microbes with monoculture-type plants and mixture microbes with mixture-type plants) and "away" combinations (monoculture microbes with mixture-type plants and mixture microbes with monoculture-type plants). When possible, the final models were simplified by pooling the last terms of the model with the residuals. Five pots were omitted from the analyses because they included two plant individuals instead of one. Statistical analyses were conducted using a software product R, version 3.0.2 (R Core Team, 2013).

Leaf chemistry

Assessment of leaf secondary metabolites and lipids — To test whether monoculture- and mixture-type plants differed in terms of secondary metabolite or lipid profiles, we assessed the profiles of leaf secondary metabolites and lipids between monoculture and mixture-type plants of *F. rubra* and *P. lanceolata* using a protocol that was adapted from the protocols developed at the Max Plank Institute of Molecular Plant Physiology (Hummel *et al.*, 2011; Giavalisco *et al.*, 2011).

HPLC grade H_2O (< 5ppb) was obtained by purification of deionized H_2O with a MilliQ gradient apparatus (Millipore, Milford, MA, USA). Acetonitrile (MeCN), isopropanol and methanol (MeOH) were all purchased from Fluka (LC-MS grade, Buchs, Switzerland), tert-butyl methyl ether (MTBE) from Sigma-Aldrich (HPLC grade, Buchs, Switzerland). As internal standards corticosterne (Sigma-Aldrich, Buchs, Switzerland), ampicilline (Fluka, Analytical Standard, Buchs, Switzerland) and 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine (Avanti, Alabaster, AL, USA) were used. Formic acid (FA) and acetic acid (AA) ULC/MS grade was bought from Biosolve BV (Valkenswaard, Netherlands) and ammonium acetate was purchased from Fluka (Buchs, Switzerland).

We collected the leaf samples at the second harvest of the present study by shock-freezing the samples in liquid nitrogen immediately after harvesting and freeze-drying the leaves for 72 h to ambient dry mass. We measured the dry mass of the samples and stored them in sealed plastic bags with silica gel at room temperature until further processing.

Prior the extraction, the leaf samples were crushed in a porcelain mortar, pre-cooled with liquid N_2, until a fine powder. Aliquots of plant powder (50 ± 2 mg) were weighed in frozen state into pre-chilled 2-ml round bottom tubes (Eppendorf, Hamburg, Germany) and stored at –80°C until the extraction.

Extraction mixture 1 (EM1), 2 µg ml-1 corticosterone in MeOH mixed with 0.67 µg ml^{-1} 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine in MTBE 1:4 (v/v), containing 0.25 µg ml^{-1} ampicilline, was pre-cooled at –20°C for 30 min. Extraction mixture 2 (EM2, H_2O/MeOH 3:1 (v/v)) was stored at ambient temperature. The EM1 (1000 µl) was added to each sample. The samples were vortexed and stored on ice for 10 min, followed by sonication in an ice cooled ultra sound bath for 10 min. After adding 500 µl EM2, the samples were vortexed and centrifuged (2 min, 14'000 rpm) at ambient temperature. The upper organic phase, containing the lipids, was split in aliquots of 2x300 µl and transferred to 1.5 ml tubes (Eppendorf, Hamburg, Germany). The remaining green, upper phase was removed with a

pipette and the samples were centrifuged (2 min, 14'000 rpm). Aliquots of the lower polar phase containing the polar metabolites were transferred to 1.5 ml tubes (2x290 µl for *P. lanceolata*, 2x250 µl for *F. rubra*). All aliquots were dried to complete dryness in a Refrigerated CentriVap Concentrator (30°C, < 10 mbar, Labconco, Kansas City, MO, US). The dry samples were stored at –20°C.

UHPLC-UV/Vis-MS experiments — The UHPLC-ESI-MS experiments were performed on a Dionex UltiMate 3000 HPLC instrument (Thermo Scientific, Germering, Germany) equipped with an autosampler, a pump and a diode-array detector (DAD) of the same producer series. The UHPLC system was connected to a QExactive Orbitrap FT mass spectrometer (Thermo Scientific, Waltham, MA, USA) equipped with an HESI source.

Analysis of secondary metabolites — The dry semi-polar fractions were re-suspended in ultrapure H_2O (*P. lanceolata* samples: 135 µl, *F. rubra* samples 116 µl), incubated for 15 min, vortexed and sonicated for 5 min at ambient temperature. After centrifugation (2 min, 14'000 rpm) the supernatant was transferred to cone-shaped glass vials (Interchroma, Zug, Switzerland). A pool of all samples analysed within the same day was prepared as quality control.

The samples were chromatographed at a flow rate of 0.4 ml min^{-1} on a RP column (Acquity UPLC HSS T3, 1.8 µm, 2.1x100 mm, Waters, Milford, MA, USA) with solvents A and B consisting of H_2O + 0.1 % FA and MeCN + 0.1 % FA, respectively. The column chamber temperature was set to 40 °C. The samples were injected at a volume of 1 µl. The gradient was isocratic at 1 % B for 1 min, went linearly from 1 to 40 % of solvent B over 10 min, linearly increased to 70 % of B in 3 min and went to 99 % of B in 2 min. The column was then washed at 99 % of B for 3 min and readjusted to 1 % of B over 3 min.

Analysis of lipids — For the lipid extract reconstruction, 100 µl 7:3 (v/v) mixture of MeCN and isopropanol was added to the dried samples. After 15 min incubation, the samples were vortexed, sonicated for 5 min and centrifuged for 2 min at 14'000 rpm. The supernatant was transferred to cone-shaped glass vials. A pool of all samples analysed within the same day was prepared as quality control.

The samples were chromatographed at a flow rate of 0.4 ml min^{-1} on a RP column (Acquity UPLC BEH C8, 1.7 µm, 2.1x100 mm, Waters, Milford, MA, USA) with solvents A and B consisting of H_2O 10 mM NH_4Ac+ 0.1 % AA and MeCN 10 mM NH_4Ac+ 0.1 % AA, respectively. The column chamber temperature was set to 60 °C. The samples were injected at a volume of 1 µl. The gradient was isocratic at 55 % of B for 1 min, went linearly from 55 to 75 % of solvent B over 3 min, linearly increases to 89 % of B in 8 min and went to 100 % of B in 3 min. The column was then washed at 100 % of B for 4.5 min and readjusted to 55 % B over 4.5 min.

MS method — For each sample, full scan MS (FS) and all ion fragmentation (AIF) were recorded alternating at 70,000 resolution. The parameters for the MS acquisition were as followed: sheath gas flow rate (N_2, 50), aux gas flow rate (N_2, 13), aux gas heater temperature (425 °C) and sweep gas flow rate (N_2, 3), S-lens RF level (55), capillary

temperature (263 °C). The AGC target setting for full scan MS experiments was set to 106 with a maximum of 30 injection times. For fragmentation NCE was set to 35.

In positive ionisation mode, the MS spectra were recorded between m/z 100 to 1500 and the spray voltage was set to 3.5 kV. In negative ionisation mode, the MS spectra were recorded between m/z 115 to 1500 and the spray voltage was set to 2.5 kV.

UV Vis absorption spectra were acquired at 254 nm and between 190 – 600 nm with 5 Hz scan rate on a diode-array detector (DAD).

Data processing — The LC-MS raw data files were analysed by using XCMS Server (Tautenhahn et al. 2012, Gowda et al. 2014). Monoculture- and mixture-type plants grown on the same soil treatment were compared in pairwise analyses with the predefined parameters of "UPLC / Q-Exactive". The mixture-type plant samples were always the control group. For both soil treatments, significant features ($p < 0.05$, $FC > 1.5$, max intensity ≤ 0, m/z tolerance 0.01, R_t tolerance 10 s) were identified with a metaXCMS analysis.

16S RNA sequencing of microbial communities

Library preparation and sequencing — To investigate whether the differences in plant growth and survival were paralleled by differences in the microbial community composition, we explored the microbiome of a subset of 150 samples (Supplemental Table S8). We therefore isolated DNA from rhizosphere soil and sequenced the 16S RNA gene sequences (Bates *et al.*, 2011). DNA was isolated from 500 mg of rhizosphere soil per plant using the FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions. We carried out targeted PCR in duplicates to amplify the variable region V4 of the prokaryotic ribosomal RNA gene using primers 515f (GTGCCAGCMGCCGCGGTAA) combined with 5' Illumina adapter, forward primer pad, and forward primer linker and barcoded 806r (GGACTACHVGGGTWTCTAAT) combined with Illumina 3' adapter, Golay barcode, reverse primer pad, and reverse primer linker (Supplemental Table S9 (Bates *et al.*, 2011). The PCR conditions for the amplification of the V4 region consisted of an initial denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 30 s, an annealing at 50 °C for 30 s, and an elongation at 72 °C for 1 min followed by a final elongation at 72 °C for 10 min. The PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). The amplicon concentrations were measured with the Fragment Analyzer and the Standard Sensitivity NGS Fragment Analysis kit (Advanced Analytical Technologies, Inc., Ankeny, IA, USA). 60 ng of each sample were pooled and paired-end sequenced (2 x 300 bp) on the Illumina MiSeq 300 system (Illumina, Inc., San Diego, CA, USA) (Beijing Genomics Institute, www.bgi.com). Short-reads were deposited at SRA (accession number SRP105254).

Identification and annotation of OTUs — Operational taxonomic units (OTUs) were generated with UPARSE (version 8.1.1861, Edgar, 2013) following the example and the tutorial given for paired-end Illumina data (drive5.com/uparse/). Reads were first quality-checked with FastQC (bioinformatics.babraham.ac.uk/projects/fastqc). Following removal of primer sequences (Supplemental Table S9) and low-quality bases with Trimmomatic (version 0.33 with the parameters ILLUMINACLIP:primerSeqs:2:30:10:8:1

SLIDINGWINDOW:5:15 MINLEN:100, Bolger *et al.*, 2014) paired-end reads were merged and filtered using usearch (with the parameters -fastq_maxdiffs 25 -fastq_maxdiffpct 10 for merging and -fastq_trunclen 250 -fastq_maxee 0.25 for filtering, Edgar, 2013). Duplicated sequences were then removed with fqtrim (version 0.9.4, Pertea, 2009). The remaining sequences were processed and clustered at 97 % sequence identity with usearch (with the parameter -minsize 2, Edgar, 2013) to obtain 10'205 OTU sequences (Supplemental File S1, Supplemental Table S11, Supplemental Table S10).

OTU sequences were annotated with the taxonomy data from SILVA (Quast *et al.*, 2013) using SINA with a minimal similarity of 90 % and the 10 nearest neighbors (www.arb-silva.de/aligner, Pruesse *et al.*, 2012). OTU abundances were finally obtained by counting the number of sequences (merged and filtered) matching to the OTU sequences (usearch with the parameters -usearch_global -strand plus -id 0.97, Edgar, 2013), Supplemental Table S10). Three samples (Sample77, Sample265, and Sample364) were removed from all further analysis because they exhibited very low counts (6, 12, and 1 counts in total). To avoid potential biases caused by plant DNA and sequencing artifacts, OTUs with similarity to chloroplast sequences (87 OTUs) and low-abundant OTUs with less than 50 counts in total or with counts in less than five samples (5'780 OTUs) were removed from the analysis (4'339 OTUs remained after this filter). From the 4'339 remaining OTUs, 3'975 and 41 were classified as bacteria and archaea, respectively (194 remained unclassified). Within the bacterial domain, the ten most abundant phyla accounted for 86.7 % of all OTUs (Supplemental Table S13). These phyla (and their representation in % of all OTUs) were Proteobacteria (35.4 %), Bacteroidetes (10.5 %), Planctomycetes (8.9 %), Chloroflexi (7.1 %), Actinobacteria (4.7 %), Verrucomicrobia (4.7 %), Acidobacteria (4.5 %), Gemmatimonadetes (4.3 %), Parcubacteria (3.3 %), and Firmicutes (3.2 %).

Data normalization and identification of differentially abundant OTUs — Variation in OTU abundance was analyzed with a general linear model in R with the package DESeq2 (version 1.14.1, (Love *et al.*, 2014) according to a factorial design with the three explanatory factors "plant history" (monoculture- and mixture-type plants), "soil treatment" (control, monoculture microbes and mixture microbes; no samples were analyzed for the fourth soil treatment with field soil), and "species" (*F. rubra*, *G. mollugo*, *G. pratense*, *L. pratensis*, *O. viciifolia*, *P. lanceolata*, *P. vulgaris* and *V. chamaedrys*) combined into a single factor (Supplemental Table S8). Specific conditions were then compared with linear contrasts (Schmid *et al.*, 2017). The four main contrasts compared (1) the two plant histories, (2) the control soil with the microbial soil treatments, (3) the two microbial soil treatments, and (4) a given plant species with all other plant species. To test for interactions, each contrast was tested across the entire data set and within the individual soil treatments or plant species. Contrasts (2), (3), and (4) were not tested separately within the two plant histories because plant history had a weak effect on the composition of the microbiomes. Within each comparison, *P*-values were adjusted for multiple testing (Benjamini-Hochberg), and OTUs with an adjusted *P*-value (false discovery rate, FDR) below 0.01 and a minimal log2 fold-change (i.e., the difference between the log2-transformed, normalized OTU counts) of 1 were considered to be differentially abundant (Supplemental Table S12). Normalized OTU counts

were calculated accordingly with DESeq2 and then log2(x+1)-transformed to obtain the normalized OTU abundances. We removed Sample 492 from all analyses because it likely represented an outlier for which the inoculation with the microbial wash failed (see Fig. 6).

Assigning OTUs to putative taxa — To better understand the differences in bacterial composition between plant species and soil treatments, we assessed the taxonomy of the significantly differentially abundant OTUs by averaging the differences in OTU abundance (i.e., log2 fold-changes) within each phylum and visualizing these differences (see Figs 8, 9, 10, Supplemental Figs S10, S11, S12).

Further data processing — To evaluate the overall differences between the microbiomes, we conducted a redundancy analysis (RDA, Oksanen *et al.*, 2017) using the normalized OTU abundances as response variables and the plant history, the plant species, the soil treatment, and all interactions as explanatory variables (Fig. 6). The two first constrained components explained 17.4 % of the overall variance and separated the control soil from the microbial soils. An exception was "Sample492", which grouped among the samples from the control soil, even though it came from a microbial soil (this sample was therefore removed as outlier from all subsequent analyses). Nonetheless, the result clearly indicated that the inoculation with soil treated with microbial filtrates provided sufficient starting material to establish distinct microbiomes.

To characterize the overall impact of plant species, plant history, and soil treatments on the bacterial community structure, we analyzed the variation in species richness (number of different OTUs) as response to the different factors. Subsequently, we tested whether bacterial species richness explained variation in plant biomass production.

Regarding the potential effect of the plant species diversity on the bacterial abundance and richness, it is important to note that the microbial soil treatment included microbial filtrates from the soil of eight plant monocultures and mixtures (one for each plant species). The average of the monoculture-microbes soil treatment thus represents a pool of eight plant species. Similarly, the average of the mixture-microbes soil treatment represents a pool of eight different plant communities (36 plant species in total, Table S1). The overall comparison across all plant species thus resembles a comparison of soil influenced by eight plant species compared to 36 plant species (without considering the interactions including more than one plant and one microbial species). The effect of the plant species diversity on the bacterial abundance may therefore be better understood through the comparisons between the two microbial soil treatments within the specific plant species.

RESULTS

Plant survival and traits

Plant survival — Out of 443 plants of the present study, 317 plants survived until the end of the experiment resulting in the survival rate of 72 %. The early survival of plants varied significantly among plant functional groups and species within functional groups (Fig. 2B), however, on average different plant histories showed equal early survival (Table 3, Fig. 2A).

The early survival of plants was lowest in control soil and highest in field soil but did not differ significantly between monoculture and mixture microbes (Fig. 2A). In particular, the control-soil treatment reduced early survival of the small herb *P. lanceolata* and the tall herb *G. mollugo* (Table 3, Fig. 2B). Field soil increased the early survival of the small herb *V. chamaedrys*, the legume *O. viciifolia* and the tall herb *G. mollugo* but reduced it in the grass *F. rubra*.

Plant functional groups and species within functional groups also varied significantly in late survival (Table 3, Fig. 2D). In addition, mixture-type plants had higher late survival than monoculture-type plants (Fig. 2C). The late survival of plants was highest in control soil but did not differ between field soil and monoculture or mixture microbes (Fig. 2C). Among the microbial soil treatments, however, plants survived significantly more often in monoculture than in mixture microbes (Fig. 2C). The two legumes *L. pratensis* and *O. viciifolia* showed increased mixture- than monoculture-type plant survival (Table 3, Fig. 2D), which mainly explained the observation of the generally higher late survival of mixture- in comparison to monoculture-type plants. Field soil reduced the late survival of grass *F. rubra* and legumes *L. pratensis* and *O. viciifolia* but not the survival of small herbs *P. lanceolata*, *P. vulgaris* and *V. chamaedrys* and tall herbs *G. mollugo* and *G. pratense* (Table 3, Fig. 2D). Whereas monoculture-type plants survived equally well in monoculture and mixture microbes, mixture-type plants showed higher late survival in monoculture microbes than in mixture microbes (Table 3, Fig. 2C). This significant overall effect was mainly caused by the increased mixture- in comparison to monoculture-type plant survival of the two legumes *L. pratensis* and *O. viciifolia* in monoculture microbes (Table 3, Fig. 2D).

Plant biomass production and biomass response to live soil treatments — The studied plant species showed an overall positive biomass response to the live soil treatments in comparison to the control-soil treatment at the first harvest (test for significance of overall mean in first row of Table 4, Fig. 3A). The biomass response to the live soil treatments varied significantly among plant functional groups (Table 4, Fig. 3B). Mixture-type plants showed a more positive biomass response than monoculture-type plants (Table 4, Fig. 3A). The plant histories did not, however, differ in terms of absolute biomass production (Table S2, Fig. S1). Increased mixture- in comparison to monoculture-type plant biomass responses were observed in *F. rubra*, *P. lanceolata*, *L. pratensis*, *O. viciifolia* and *G. mollugo* whereas the opposite was observed in *P. vulgaris*, *V. chamaedrys* and *G. pratense* (Fig. 3B). In addition, biomass responses to the field soil were significantly stronger than biomass responses to monoculture or mixture microbes (Table 4, Fig 3A).

Weaker plant biomass responses to live soil treatments were observed at the second harvest (Fig. 3C). Differences among plant functional groups still explained a significant part of the variation in biomass responses (Table 4, Fig. 3D). Mixture-type plants of *F. rubra*, *V. chamaedrys*, *L. pratensis*, *O. viciifolia*, *G. mollugo* and *G. pratense* showed more positive biomass responses than monoculture-type plants whereas the opposite was the case for *P. lanceolata* and *P. vulgaris* (Fig 3D), which resulted in an overall more positive biomass response of mixture-type plants in comparison to monoculture-type plants (Table 4, Fig. 3C). Monoculture-type plants showed negative biomass responses to monoculture microbes whereas the other three combinations monoculture-type plants with mixture microbes and

mixture-type plants with monoculture or mixture microbes all showed positive responses (significant interaction in last row above residuals in Table 4, Fig. 3C), indicating that co-evolution potentially increased the pathogenicity of monoculture microbes for monoculture-type plants.

Leaf damage — Plant leaf damage caused by common glasshouse pests varied significantly among plant functional groups and species within functional groups (Table 5, Fig. 4). Mixture-type plants generally had more leaf damage than monoculture-type plants, but this was not the case in the small herb *V. chamaedrys*, the legume *L. pratensis* and the tall herb *G. pratense* (Table 5, Fig 4).

AMF colonization probability and colonization rate — 97 % of the plants in the field-soil treatment showed the presence of AMF colonization in the roots (Fig. 5A). We observed failures of AMF colonization only in *O. viciifolia* of which 25 % of the monoculture- and 50 % of the mixture-type plants were not visibly colonized (Fig. 5A). AMF colonization rate varied among plant functional groups and species within functional groups (Table 6, Fig. 5B). Higher AMF colonization of mixture-type plants in comparison to monoculture-type plants was observed in the grass *F. rubra*, the small herbs *P. lanceolata* and *P. vulgaris* and the legume *L. pratensis* whereas the opposite was observed in the small herb *V. chamaedrys*, the legume *O. viciifolia* and the tall herbs *G. mollugo* and *G. pratense* (Table 6, Fig 5B).

Plant morphological traits — All measured plant traits varied significantly among plant functional groups and species within functional groups except for LDMC, which only showed marginally significant variation between species within functional groups. Apart from *L. pratensis* and *G. mollugo*, the studied species showed higher leaf absorbance in monoculture- than in mixture-type plants (Table S3, Fig. S2). The small herbs *P. lanceolata*, *P. vulgaris* and *V. chamaedrys* and the tall herbs *G. mollugo* and *G. pratense* had lower leaf absorbance in field soil than in soil treatments with monoculture or mixture microbes whereas the opposite was the case for the legumes *L. pratensis* and O. *viciifolia* (Table S3, Fig. S2B).

Monoculture-type plants generally had higher LDMC than mixture-type plants (Fig. S3A), but this was not the case for the two tall herbs *G. mollugo* and *G. pratense* (Table S4, Fig. S3B). In field soil, monoculture-type plants of the small herbs *P. lanceolata*, *P. vulgaris* and *V. chamaedrys* and the legumes *L. pratensis* and *O. viciifolia* had higher LDMC than mixture-type plants whereas the grass *F. rubra* and the tall herbs *G. mollugo* and *G. pratense* showed the opposite (Table S4, Fig. S3B). Apart from tall herbs *G. mollugo* and *G. pratense*, monoculture-type plants also had generally higher LMA than mixture-type plants (Table S5, Fig. S4).

In terms of maximum height, mixture-type plants of the grass *F. rubra* and the small herbs *P. lanceolata*, *P. vulgaris* and *V. chamaedrys* were smaller and mixture-type plants of the legumes *L. pratensis* and *O. viciifolia* and the tall herbs *G. mollugo* and *G. pratense* were taller than monoculture-type plants (Table S6, Fig. S5), which indicates that increased height differences between short and tall species may have evolved in species mixtures, consistent

with earlier findings after 8-years of selection in the same experiment (Zuppinger-Dingley *et al.*, 2014).

As expected due to the sterilization of soil biota, plants in the control-soil treatment showed overall lower root damage rates than in live soil treatments (Table S7, Fig. S6A). Monoculture-type plants showed higher root damage than mixture-type plants in the grass *F. rubra*, the small herb *V. chamaedrys*, the legumes *L. pratensis* and *O. viciifolia* and the tall herb *G. pratense* whereas the opposite was observed for the small herbs *P. lanceolata* and *P. vulgaris* and the tall herb *G. mollugo* (Table S7, Fig. S6B).

Leaf chemistry

Monoculture- and mixture-type plants of *F. rubra* produced separate clusters in the principal component analysis of secondary metabolites in the negative but not in the positive ionization mode (Fig. S7). Differential clustering between monoculture- and mixture-type plants of *F. rubra* was not observed in the case of lipids (Fig. S7). Monoculture- and mixture-type plants of *P. lanceolata* did not show differential clustering in principal component analyses for secondary metabolites and lipids (Fig. S8). Differentially regulated metabolic features of secondary metabolites and lipids between monoculture- and mixture-type plants were more often unique to the plants that were grown in the control- or the field-soil treatments than common to plants grown in different soil treatments, suggesting that the soil treatments altered the production of foliar secondary metabolites and lipids (Fig. S9). Higher numbers of foliar secondary metabolites than lipids were found to be common to plants regardless of the soil treatments they were grown on, suggesting that secondary metabolites more often than lipids were unique to the plant histories.

16S sequencing of microbial communities

Plant species, soil treatments and their interaction explained a significant part of variation in bacterial species richness (Table 7, Fig. 7). Bacterial species richness was significantly higher in microbial soil treatments compared to control soil (Table 7, Fig. 7A). Monoculture and mixture microbes did not show overall difference in bacterial species richness but did so in interaction with plant species: mixture microbes had higher species richness than monoculture microbes in the grass *F. rubra*, the small herb *P. lanceolata* and the legume *O. viciifolia* whereas the opposite was the case for the legume *L. pratensis* and the tall herb *G. pratense* (Table 7, Fig. 7B). This indicates that the overall bacterial community structure was primarily determined by plant species and soil treatment.

Bacterial species richness did not explain variation in plant biomass production at the first harvest if fitted after plant species (plant species: $P < 0.001$, SS = 35.27), but did so in interaction with plant species ($P = 0.03$, SS = 7.28 %). Fitted after plant species, bacterial species richness had a significant influence on plant biomass production at the second harvest (plant species: $P < 0.001$, bacterial species richness: $P = 0.018$) but it explained only little of the overall variation (plant species SS = 78.13 %, bacterial species richness SS = 0.87 %). These results indicate that differential effects of monoculture and mixture microbes on species-specific plant growth (see Table 4) may be related to differences in richness and composition of microbial communities.

To identify OTUs which contributed to the differences in the microbial community structures, we tested each OTU for differential abundance between the different plant histories, plant species, and soil treatments. We therefore combined the three experimental treatments into a single factor and compared specific conditions with linear contrasts (see Methods for details). Out of the 4'339 OTUs tested, 2'091 were significant in at least one treatment comparison using contrast analysis (Table 8). The contrasts comparing the plant histories across the entire data set, within specific soil treatments or within specific plant species were almost never significant (less than 13 OTUs in any case), suggesting that monoculture- and mixture-type plants generally could not accumulate different microbial communities in their rhizosphere during the five months of growth in the glasshouse. In contrast, comparisons between the different soil treatments were often significant (e.g., 972 OTUs were different between the control soil and the microbial soil treatments). Likewise, contrasts comparing one plant species to all other plant species were frequently significant as well (e.g., 498 OTUs were different between *F. rubra* and all other plant species). In agreement with the results of bacterial species richness, the number and the identity of the OTUs identified as significantly differentially abundant between soil treatments and plant species varied if they were tested within a given plant species or soil treatment, respectively (i.e., there was an interaction between soil treatment and plant species, Figs 8, 9, 10; Supplemental Figs S10, S11, S12). These results demonstrate that the sampled microbiomes of rhizosphere soils have mainly been shaped by the previous long-term (11 years) "soil-training" by monocultures or mixtures of particular plant species and not by the plants grown in the inoculated soils during the test phase of the glasshouse experiment (see Fig. 11) for a visualization of the distances between the individual samples using the normalized abundances of the 2'091 significant OTUs).

Differences between monoculture microbes and mixture microbes were highly specific to the plant species (Fig. 9). In total, 844 OTUs were significantly differentially abundant between the two microbial soils if tested separately for each plant species. The majority of them (566 OTUs) were unique to a specific plant species. Likewise, only 137 significant OTUs were identified if tested across all plant species (out of which 23 were not identified in the plant species-specific comparisons). In contrast, 73.9 % of all OTUs identified as differentially abundant between the control soil and the two microbial soil treatments, were also significant if tested across all plant species (972 out of 1'316 OTUs). On average, per plant species, 91 and 64 OTUs were more abundant in mixture microbes and monoculture microbes, respectively. Except for *G. pratense*, mixture microbes always had a higher number of OTUs with increased abundance than monoculture microbes. This was also true if tested across all plant species, where 105 and 32 OTUs exhibited increased abundance in the mixture microbes and monoculture microbes, respectively (Table 8). It is noteworthy, that mixture microbes of *G. pratense* originated from a field plot with tall herbs whereas all other mixture microbes originated from plant communities with a minimum of two plant functional groups (Table S1). Taken together, bacterial abundance was generally higher in mixture microbes than in monoculture microbes, suggesting that plant species diversity generally increased the abundance of soil bacteria, whereas it did not generally increase their richness (see above). When bacterial richness in monoculture vs. mixture microbes was compared by

only including the significantly differentially abundant bacterial OTUs to the analysis, bacterial richness, however, correlated with bacterial abundance (Table S14).

The exception of *G. pratense* might indicate that this effect may also depend on the plant functional groups and not just the plant species identity. However, this requires further investigation.

As expected, most phyla were more abundant in the microbial soil treatments than in the control-soil treatment. This pattern was stable across the different plant species, and overall, only *Firmicutes*, *Saccharibacteria*, and *Chlorobi* were more abundant in the control-soil treatment than in the microbial soil treatments (Fig. 8). More specifically, *Firmicutes* and *Chlorobi* included bacteria from the classes *Clostridia* and *Bacilli* (mostly *Paenibacillus*), and *Chlorobia*, respectively. Bacteria within the classes *Clostridia* and *Bacillia* can form endospores and are obligate and facultative anaerob, respectively (Tracy *et al.*, 2008). Likewise, *Chlorobia* are obligate anaerobic bacteria (Eisen *et al.*, 2002). Some *Clostridia* can fix nitrogen (Elbadry *et al.*, 1999), *Chlorobia* oxidize sulfur, which is important for plant growth (Freney, 1967), and several *Paenibacillus* species serve as plant growth-promoting rhizobacteria (Ahemad & Kibret, 2014). Thus, these bacteria might have influenced plant biomass production, and the effect was likely to be positive. In microbial soil treatments, plant biomass production might have been promoted by ammonia-oxidizing microbes belonging to the phyla *Nitrospirae* (exclusively *Nitrospira*) and *Thaumarchaeota* (Fig. 8). *Thaumarchaeota* oxidize ammonia into nitrite (Hatzenpichler, 2012), and *Nitrospira* can perform complete oxidation into nitrate (Daims *et al.*, 2015). Nitrification can also be carried out by members of the family *Nitrosomonadaceae* (phylum *Proteobacteria*, (Prosser *et al.*, 2014)), for which only one OTU was overall significant and more abundant in microbial soils (a member of the genus *Nitrosomonas*). Interestingly, each significant OTU belonging to either *Nitrospirae, Thaumarchaeota,* or *Nitrosomonadaceae* was overall more abundant in the microbial soils compared to the control soil. We therefore speculate that the increased biomass production of plants in the microbial soil treatments might have been related to the higher abundance of these nitrifying microbes. However, further positive or negative effects of the microbiomes on plant growth could not be excluded.

As shown above, monoculture microbes reduced the biomass response of monoculture-type plants but not the biomass response of mixture-type plants in comparison to plant biomass production in the control-soil treatment. To identify the cause of the differential biomass response of the two plants histories to monoculture microbes, we analysed differentially abundant OTUs in the rhizosphere of monoculture- and mixture-type plants grown in monoculture microbes. Out of six OTUs that were significantly more abundant in the rhizosphere of monoculture-type plants, one was isolated from the rhizosphere of *L. pratensis* and identified as non-pathogenic *Prosthecobacter* (e.g. Hedlund *et al.*, 1997; Yoon, 2014), and five were isolated from rhizosphere of *P. vulgaris* monoculture-type plants which, in contrast to the majority of the studied species, showed particularly positive biomass response to monoculture microbes. Thus, we could not identify the cause of negative biomass response of monoculture-type plants to monoculture microbes.

Next, we tested whether monoculture microbes in comparison to mixture microbes were enriched for bacteria antagonizing plant pathogens. We therefore extracted all OTUs annotated with taxa matching the plant growth promoting microbes listed in Ahemad &

Kibret (2014) and assessed whether these OTUs were (significantly) differentially abundant between the microbial soil treatments (Fig. 12). We did not observe a consistent pattern across plant species. Given a specific plant species, microbial taxa with species known to antagonize plant pathogens appeared to be slightly enriched in monoculture microbes. *Pseudomonas*, *Rhizobium*, *Azospirillum*, and *Streptomyces* were frequently more abundant in monoculture microbes. However, OTUs classified as *Mesorhizobium*, *Bacillus*, or *Paenibacillus* were more abundant in mixture microbes (Fig. 12).

Considering that the effect may depend on the differential abundance of the entire taxa (instead of individual OTUs), we also summarized the differences in abundance across the taxa, irrespective of the significance of the individual OTUs (Fig. 13). The results were similar to the analysis with the individual OTUs, with the average difference in taxon abundance between the microbial soils being highly specific to the plant species.

DISCUSSION

In the present study, we tested whether plants and soil microbes that had been co-selected over 11 years in plant monocultures vs. mixtures would show differences in plant phenotypes and microbiome compositions when tested in different combinations. To increase generality, we did these tests for eight plant species belonging to four functional groups. Regarding the plants, we hypothesized that selection in monocultures favours plants that compromise biomass production for better defense against pathogenic soil microbes, which are potentially accumulating under these conditions (Petermann *et al.*, 2008; Schnitzer *et al.*, 2011). Alternatively, plants that associate with beneficial soil microbes may experience a selection advantage in monocultures. Regarding the soil microbes, we hypothesized that the different plant species could assemble distinct microbiomes in their rhizosphere soils and that these differed between plant monocultures and mixtures. Finally, we hypothesized that co-adaptation between plants and microbiomes could lead to increased or decreased plant performance in "home"-combinations (monoculture-type plants with monoculture microbes and mixture-type plants with mixture microbes) as compared with "away"- combinations (monoculture-type plants with mixture microbes and mixture-type plants with monoculture microbes). We tested our hypotheses by growing monoculture- and mixture-type plants of the eight species in the presence and absence of monoculture or mixture microbes and in soils including the full community of co-selected soil organisms.

Monoculture-type plants showed lower average biomass responses than mixture-type plants to live soil treatments throughout the experiment. Biomass production, however, did not vary significantly between the two plant-selection histories (compare Figs 3A and 3C vs. Figs S1A and S1C). This suggested that the differences in biomass responses between monoculture- and mixture-type plants were initiated by differential responses of the plant histories to the soil treatments of the present study rather than by the hypothesized growth trade-offs of monoculture-type plants in comparison to mixture-type plants. Indeed, relative to mixture-type plants, monoculture-type plants of the majority of studied plant species produced higher, albeit not significantly higher, biomass than mixture-type plants in the control-soil treatment, which reduced the overall biomass response to live soil treatments of

monoculture-type plants in comparison to mixture-type plants at both harvests. Taken together, these results do not support our hypothesis that monoculture-type plants have been selected for greater growth trade-offs in comparison to mixture-type plants. Instead, the results suggest that the feedbacks of soil organisms are more positive to the growth of mixture-type plants than the growth of monoculture-type plants.

Nevertheless, we observed lower average leaf damage in monoculture-type than in mixture-type plants, supporting the hypothesis that monoculture-type plants should invest more resources for plant defense. Monoculture-type plants of the majority of the studied species had, in addition, significantly increased LDMC and LMA in comparison to mixture-type plants. Increased values of LDMC and LMA are closely related to the increased toughness, or hardness, of leaf tissues (Hanley *et al.*, 2007; Pérez-Harguindeguy *et al.*, 2013), which reduces the digestibility of leaf tissues thereby protecting plants from herbivory (Turner, 1994). Increased leaf toughness has also been suggested to positively correlate with an accumulation of defense compounds in leaves (Coley, 1988). Higher LDMC and LMA in monoculture- than mixture-type plants could, therefore, provide an explanation for the increased defense of monoculture-type plants. The also observed higher root damage in mixture- than in monoculture-type plants of five of the eight species further supports the hypothesis of increased defense, at least for these species. Increased LDMC and LMA are additionally related to higher resource investment in leaves and longer foliar life span (Turner, 1994; Wilson *et al.*, 1999; Westoby *et al.*, 2002). Increased LDMC and LMA of monoculture- compared to mixture-type plants therefore additionally suggest that monoculture-type plants allocate more resources to leaves than mixture-type plants. The view of increased resource investment to leaves by monoculture-type plants was also supported by our observation that monoculture-type plants of the majority of the studied species had increased leaf absorbance in comparison to mixture-type plants. Increased leaf absorbance, as well as higher LMA, have been shown to correlate with a higher area-based content of nitrogen (Niinemets, 1997; Moran *et al.*, 2000). Together these results suggest that monoculture- and mixture-type plants may have been selected for differential resource-use strategies over the course of the selection although we did not find evidence for an increased growth–defense trade-off of monoculture-type plants regarding biomass production.

The analyses of foliar secondary metabolites of *F. rubra* and *P. lanceolata* did not show a clear indication that monoculture- in comparison to mixture-type plants would be better defended. Plants, however, produce secondary metabolites also for many other purposes than defense (Bourgaud *et al.*, 2001). The finding that monoculture- and mixture-type plants did not differ in terms of secondary metabolite profiles does not, therefore, mean that the plants would not differ in terms of defense-specific secondary metabolites. In the present study, we analysed the profiles of plant secondary metabolites without specifically focusing on defensive secondary metabolites. Further research on plant species-specific defense-related compounds (see e.g. Marak *et al.*, 2002) is required to understand why monoculture-type plants were less damaged than mixture-type plants.

Although monoculture- and mixture-type plants did not differ in early survival, mixture-type plants showed significantly increased late survival in comparison to monoculture-type plants. This effect was mainly caused by the particularly low late survival of monoculture-type plants of the legumes *L. pratensis* and *O. viciifolia* in comparison to

mixture-type plants of the same species. In the case of legumes, the results thus conflicted with our hypothesis that monoculture-type plants would be better defended than mixture-type plants and also with the observation that, in comparison to mixture-type plants, monoculture-type plants of the two leguminous species were less damaged (see Fig. 4). It is possible, however, that the monoculture-type plants of *L. pratensis* and *O. viciifolia* that survived until the end of the experiment were better defended than the ones that died, as suggested in Chapter 2 of this thesis.

Regarding plant height, we observed mixture-type plants of short species to be shorter and tall species to be taller than the corresponding monoculture-type plants in the present study. Selection for increased niche complementarity between co-existing plant species has been considered as one of the potential mechanisms explaining why diverse plant communities become more productive over time (Hector *et al.*, 1999; Loreau, 2000). A recent finding showed that increased height differences between co-existing plants in species mixtures can be one mechanism to increase niche complementarity (Zuppinger-Dingley et al. 2014). Increased height differences of mixture- in comparison to monoculture-type plants observed in the present study support this view.

The microbial and control-soil treatments were clearly distinguished from each other by a differential clustering in the redundancy analysis of bacterial OTUs (see Fig. 6). In addition, microbial soil treatments showed significantly higher bacterial species richness than the control-soil treatment (Fig. 8). These results confirmed that the isolation and trap-culturing of soil microbes and the inoculation of the soil treatments were successful in the present study. Some bacteria, especially those with ability to live in anaerobic conditions and to produce endospores, were more abundant in control than in the microbial soil treatments. Considering the properties of these abundant bacteria in the control soil it is possible, albeit unlikely, that some of the bacteria had survived the autoclaving of the soil inocula and thus got a head start in the invasion of the autoclaved soil inoculum due to a founder effect. Among the abundant bacteria in the control-soil treatment we also found groups with plant growth-promoting properties and the ability to improve plant sulphur acquisition suggesting that these bacteria might have improved plant biomass production in the control-soil treatment during the experiment.

Mixture microbes showed higher bacterial abundance than monoculture microbes in seven out of eight plant species of the present study. Mostly, higher abundance in mixture microbes meant that the specific bacterial OTU was present in mixture microbes but absent in monoculture microbes, suggesting that not only bacterial abundance but also richness was higher in mixture than monoculture microbes. In contrast, only three of the eight studied plant species showed increased bacterial richness of mixture in comparison with monoculture microbes when the absolute numbers of detected OTUs were analysed within plant species (see Fig. 7B). However, when we only included significantly differentially abundant bacterial OTUs, we observed bacterial richness to correlate with bacterial abundance. Bacterial abundance and richness were higher in mixture than monoculture microbes in seven species whereas the opposite was the case for one species, the tall herb *G. pratense* (see Table S14). Previous studies also found evidence for a positive correlation between plant diversity and soil bacterial abundance (Stephan *et al.*, 2000; Eisenhauer *et al.*, 2017), or generally soil microbial abundance (Eisenhauer *et al.*, 2013; Thakur *et al.*, 2015). Studies examining the

correlation of plant species diversity and soil bacterial richness have varied in their outcome from positive (Stephan *et al.*, 2000; Garbeva *et al.*, 2006) to negative correlation (Schlatter *et al.*, 2015) to the absence of correlation (Dassen *et al.*, 2017). Because the habitats and resources in the rhizosphere tend to vary between different plant species (Hooper *et al.*, 2000; Berg & Smalla, 2009; Eisenhauer *et al.*, 2017), increasing plant species diversity could provide larger variety of resources and habitats for microbes and thereby explain the higher bacterial abundance and richness of mixture microbes than monoculture microbes observed in the present study. Additionally, the clear differences in bacterial composition between the soil treatments originating from monocultures and mixtures, and the weak influence of plants history (see Fig. 11), indicate that the differences in bacterial abundance and diversity between the monoculture and mixture microbes did not develop during the five months of trap-culturing but had already been developed at the field site. Our results, thus, illustrate that the amount of starting material used in the establishment of trap-cultures was sufficient for bacterial abundance and diversity to be maintained during the five months of trap-culturing at least to some extent.

The only case in which bacterial abundance and richness of monoculture microbes exceeded mixture microbes was observed for the tall herb *G. pratense*. In this case, the plant species of the mixture all belonged to the same plant functional group. For all other plant species, the plant species in the mixtures belonged to at least two plant functional groups (see Table S1) and bacterial abundance and richness was higher for mixture than for monoculture microbes. Previous studies have shown that plant functional groups influence the bacterial abundance (Stephan *et al.*, 2000; Bartelt-Ryser *et al.*, 2005; Latz *et al.*, 2012, 2016; Lange *et al.*, 2014) and richness (Stephan *et al.*, 2000; Dassen *et al.*, 2017) in soil, and plant functional groups have recently been suggested to be even more important determinants of bacterial richness than plant species identities (Dassen *et al.*, 2017). Taken together, the results of the present study suggest that bacterial abundance and richness in the rhizosphere generally increase with increasing plant species diversity, but that they are also positively influenced by plant functional diversity.

In comparison to mixture microbes, monoculture microbes had an equal influence on early plant survival but more positive influence on late plant survival. Especially mixture-type plants showed increased late survival in monoculture microbes. These effects were mainly caused by the legumes *L. pratensis* and *O. viciifolia* that showed higher mixture-type plant than monoculture-type plant late survival especially in monoculture microbes. Thus, in the case of legumes, the results provided support for our hypothesis that "home" and "away" combinations of plants and soil microbes lead to differential outcomes in terms of plant performance. Apart from the legumes, monoculture and mixture microbes did not cause differences to the survival of monoculture- and mixture-type plants. These results thereby suggest that the influence of soil organisms on plants differ among plant functional groups.

Monoculture microbes reduced the average biomass response of monoculture-type plants but not the average biomass response of mixture-type plants at the second harvest in the present study, which contradicted our hypothesis that monoculture-type plants would be better defended against specialized pathogens than mixture-type plants. Instead, this result represents a case where co-adaptation between plants and co-selected soil microbes led to decreased rather than increased plant performance. We had expected the specialized

pathogens isolated from the soil of plant monocultures to be more detrimental to mixture-type plants, which we hypothesized to invest less resources in pathogen defense. Our results supported earlier findings that specialized pathogens accumulate in monocultures over time (Petermann *et al.*, 2008; Eisenhauer *et al.*, 2012; van der Putten *et al.*, 2013). In terms of the specificity of the accumulating pathogens, however, our results do not support previous views: the result that monoculture-type plants showed reduced biomass response with monoculture microbes, whereas mixture-type plant did not, suggests that long-term selection of plants in monocultures has led to an accumulation of pathogens that are rather plant genotype-specific than species-specific. The influence of monoculture microbes on the biomass response of monoculture-type plants was weaker at the first harvest (compare Fig. 3A vs. 3C), suggesting that the establishment of soil microbial communities from inoculum requires time. Taken together, our findings indicate that soil communities sampled from plant monocultures had become enriched with specific enemies of monoculture-type plants during the 11 years of co-selection.

Monoculture- and mixture-type plants generally showed more positive biomass responses to the field-soil treatment than to the soil treatments with monoculture or mixture microbes (see Fig. 3). Furthermore, the majority of the studied plant species showed the highest survival in field soil in comparison to the other soil treatments. The field-soil treatment included mycorrhizal fungi whereas soil treatments with monoculture or mixture microbes consisted of a simplified soil community without organisms larger than 25 µm in diameter. Given the ability of AMF to increase plant biomass production and protect plants from the detrimental effects of soil pathogens (Smith & Smith, 2011) and the 97 % AMF colonization probability found in the present study among the plants grown in the field-soil treatment (see Fig. 5A), it is likely that AMFs were responsible for the observed promotion of plant biomass production in the field-soil treatment in comparison to the soil treatments with mixture and (for monoculture-type plants especially) monoculture microbes. AMF have previously been shown to promote the establishment of seedlings for instance by improving nutrient supply (van der Heijden, 2004), suggesting that the observed increase in plant survival was also promoted by the presence of AMF. Considering that the field-soil treatment represented a full soil community, it presumably differed from the other soil treatments also by a larger soil biodiversity (diversity and abundance of soil organisms). Higher soil biodiversity has been shown to improve the functioning of ecosystems in general by promoting multiple crucial ecosystem processes such as decomposition and nutrient cycling at the level of plant communities (e.g. Wagg *et al.*, 2014). Similarly, higher biodiversity of soil organisms in the field-soil treatment in comparison to the other soil treatments in the present study could have promoted such processes, thereby additionally promoting plant growth and survival. The positive influence of soil biodiversity on plant survival and biomass production was also supported by the observation that plant survival and biomass production in microbial soil treatments were lower than in the field-soil treatment but higher than in the control-soil treatment at the first harvest.

Monoculture-type plants, however, clearly did not experience more positive feedbacks from the co-selected full soil communities of the field-soil treatment than was the case for mixture-type plants. Previous findings from the same biodiversity experiment after 8 years of co-selection indicated that monoculture-type plants had developed positive plant–soil

feedbacks (Zuppinger-Dingley et al. 2016). In that experiment, mixture-type plants were also tested with monoculture microbes and showed negative plant–soil feedbacks. Our results, thus, did not support the previous finding by Zuppinger-Dingley et al. (2016) and also not our hypothesis that plants associating stronger with beneficial soil microbes would experience a selection advantage in monoculture. Because the present experiment consisted of individually grown plants, whereas Zuppinger-Dingley *et al*. (2016) studied communities of plants, the differential results may have been caused by the absence of plant–plant interactions in our experiment and the presence of them in the study of Zuppinger-Dingley *et al.* (2016). Considering that monoculture-type plants showed a more positive biomass response to the field soil than to the simplified monoculture microbes, our results suggest, in agreement with previous research (Eisenhauer *et al.*, 2012; van der Putten *et al.*, 2013), that higher soil biodiversity can protect plants from the detrimental effects of specialized enemies.

Although the biomass response of monoculture- and mixture-type plants to monoculture microbes differed significantly, the bacterial abundance in the rhizosphere of the two plant selection histories grown with monoculture microbes varied very little. Within the subset of plants that were grown with monoculture microbes, only six OTUs were more abundant in the rhizosphere of monoculture-type plants of which five were observed from the plants of *P. vulgaris* with particularly positive growth-response and one observed from *L. pratensis* was belonging to the group of non-pathogenic bacteria. Thus, our results suggest that the observed growth reduction of monoculture-type plants with monoculture microbes may not have been caused by bacteria. A large group of soil microbes have the ability to influence plant growth negatively. Besides bacteria, such microbes include fungi, viruses and water moulds (Bever *et al.*, 2015), and from the size point of view they would have been included in the soil treatments with monoculture (or mixture) microbes if present in the originally sampled soil. Thus, further research is required to understand the potential role of other soil pathogens in the reduced biomass response of monoculture-type plants in response to monoculture microbes.

Conclusions

The results of the present study supported our hypothesis that co-selection of plants and soil microbes in plant monocultures and mixtures leads to differences in plant phenotypes and microbial composition. We found evidence for the hypothesis that monoculture-type plants have been selected for increased allocation of resources to defense. We did not, however, find evidence for an increased growth trade-off of monoculture- in comparison to mixture-type plants, suggesting that the increased investment of resources for defense was not strong enough to cause significant differences in biomass production. In addition, mixture-type plants showed increased height variation in comparison to monoculture-type plants, suggesting, in agreement with previous research (Zuppinger-Dingley et al. 2014), that mixture-type plants were selected for better combining ability in comparison to monoculture-type plants.

We observed particularly strong negative effects of co-selected soil microbes on the biomass production of monoculture-type plants. Higher soil biodiversity, however, protected monoculture type plants from these negative effects. These results suggest that selection in monocultures may promote an accumulation of pathogens that are rather genotype-specific

than species-specific. Because intensive agriculture tends to reduce the soil biodiversity of cropping systems (Tsiafouli *et al.*, 2015), such genotype-specific pathogens may also reduce yields of agricultural monocultures.

Finally, the results of the present study demonstrate that changes in plant phenotypes, soil microbial composition and plant–microbe associations in response to local plant species diversity may occur over ecological time-scales. These findings suggest that high biodiversity both below and above ground may provide important insurance for plant biomass production in the long term. Furthermore, these results emphasize that concerns about plant biodiversity are also concerns about soil biodiversity.

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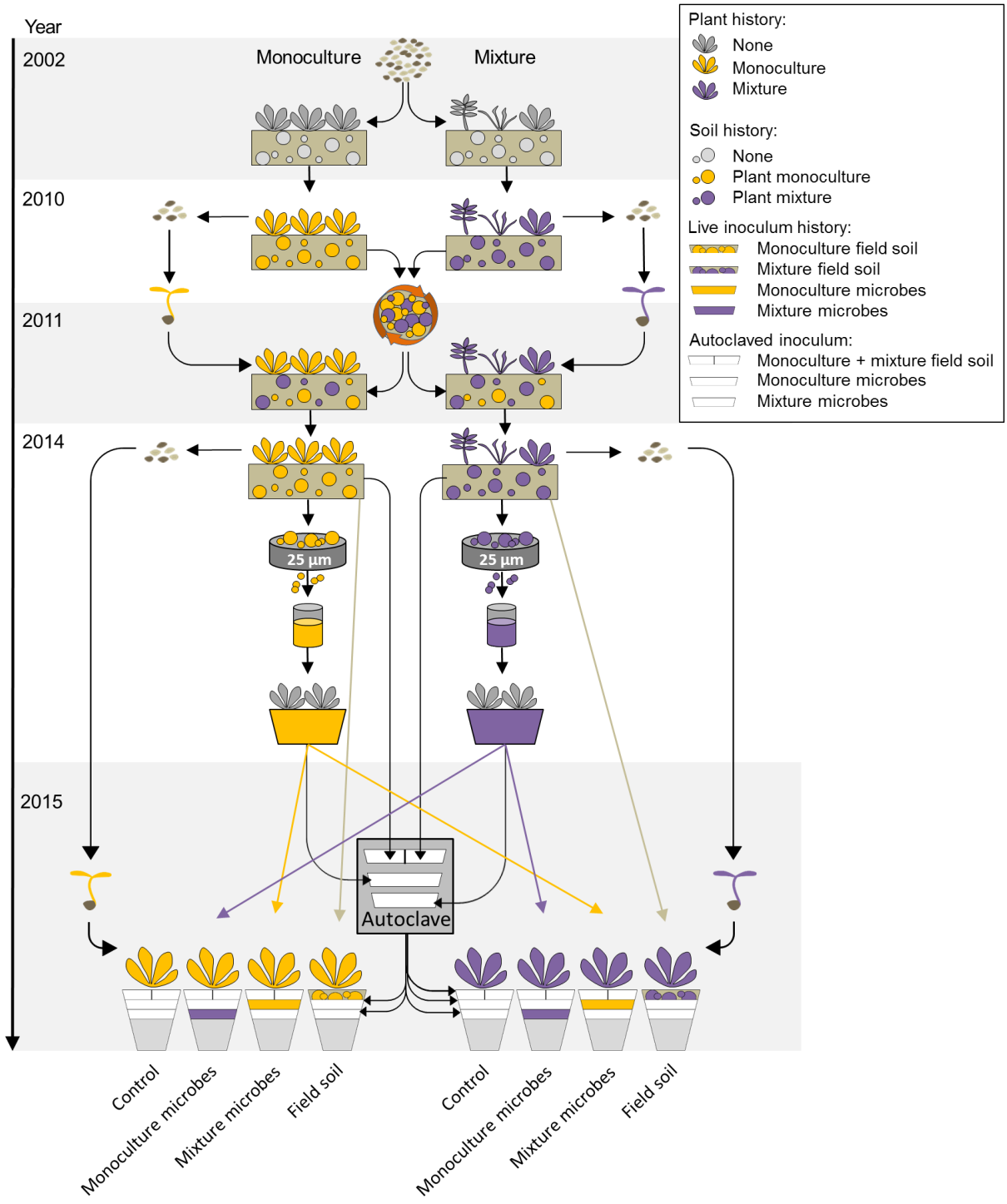


Figure 1. Experimental design. Plant monocultures and mixtures were sown in the Jena Experiment in 2002 and maintained until 2010. In 2010, plants of 48 plots were taken to the first controlled reproduction and the soil of the plots was pooled, mixed and placed back to the same locations. In spring 2011, seeds of the first controlled reproduction were germinated and planted to the mixed soil in the Jena Experiment in identical plot locations and species composition as their parents were maintained. Plant communities were maintained for additional three years to allow re-association and potential co-assembly and co-evolution with the co-selected soil organisms. In spring 2014, plants were taken to a second controlled

seed production, their rhizosphere soil was stored and part of it wet sieved to isolate microbes smaller than 25 µm in diameter and to thus exclude arbuscular mycorrhizal fungi from the soil microbiota. The isolated microbes were allowed to accumulate in trap-cultures for five months with trap plants that did not share a community-selection history with the microbes. To establish the soil treatments of the present study, we filled pots with sterile soil and added 9 % live inoculum of either microbes isolated from plants grown in monocultures (monoculture microbes), microbes isolated from plants grown in mixtures (mixture microbes) or a pool of freshly collected and stored home field home soil of the plant to be planted to the pot (field soil). To standardize the nutrient composition between pots, we added an autoclaved counterpart of the remaining inocula to each pot. The control-soil treatment received the same amount of each inocula but all inocula were autoclaved. Finally one monoculture-type plant or mixture-type plant, germinated from the seeds of second controlled seed production, was planted to each pot. Our main hypothesis was that specialized pathogens among monoculture microbes have the most negative effect on plant growth. We hypothesized that, in comparison to mixture-type plants, monoculture-type plants are either selected for better defense against the negative effects of monoculture microbes or are better protected from the negative effects by co-selected beneficial microbes. Monoculture microbes reduced the growth of monoculture-type plants but not the growth of mixture-type plants in the present experiment. Thus, we found no support for the hypothesis that monoculture-type plants are better defended against monoculture-microbes, instead, pathogens among monoculture-microbes were more specialized to monoculture-type plants than we expected. We observed that monoculture-type plants were less negatively influenced by the specialized pathogens in the presence of AMF (the field-soil treatment) suggesting that AMF may counter-act the negative effects of specialized soil pathogens in monocultures.

Table 1. Number of monoculture- and mixture-type plant replicates of four functional groups and eight species grown on the soil treatments of the experiment.

			Soil treatments			
Species	Functional group	Plant history	Control	Monoculture microbes	Mixture microbes	Field soil
F.rubra	Grass	Monoculture	7	7	7	7
		Mixture	7	7	7	7
G.mollugo	Tall herb	Monoculture	7	7	7	7
		Mixture	7	7	7	7
G.pratense	Tall herb	Monoculture	7	7	7	7
		Mixture	7	7	7	7
L.pratensis	Legume	Monoculture	7	7	7	7
		Mixture	7	7	7	7
O.viciifolia	Legume	Monoculture	7	7	7	7
		Mixture	7	7	7	7
P.lanceolata	Small herb	Monoculture	7	7	7	7
		Mixture	7	7	7	7
P.vulgaris	Small herb	Monoculture	7	7	7	7
		Mixture	7	7	7	7
V.chamaedrys	Small herb	Monoculture	7	7	7	7
		Mixture	7	7	7	7
					448	

Table 2. Plant traits measured during the experiment.

Measured plant trait	Unit
Aboveground biomass, first harvest	g dry weight/pot
Aboveground biomass, second harvest	g dry weight/pot
Leaf damage	rate 0–5 (none to high)
AMF colonization probability	presence/absence
AMF colonization rate	%
Leaf absorbance	SPAD
Leaf dry matter content (LDMC)	$mg\ g^{-1}$
Leaf mass per area (LMA)	g/cm^2
Maximum height	cm
Root damage	%

Notes: SPAD values are index values, defined by the manufacturer of the chlorophyll content measuring device, that indicate the relative amount of chlorophyll present in the leaf.

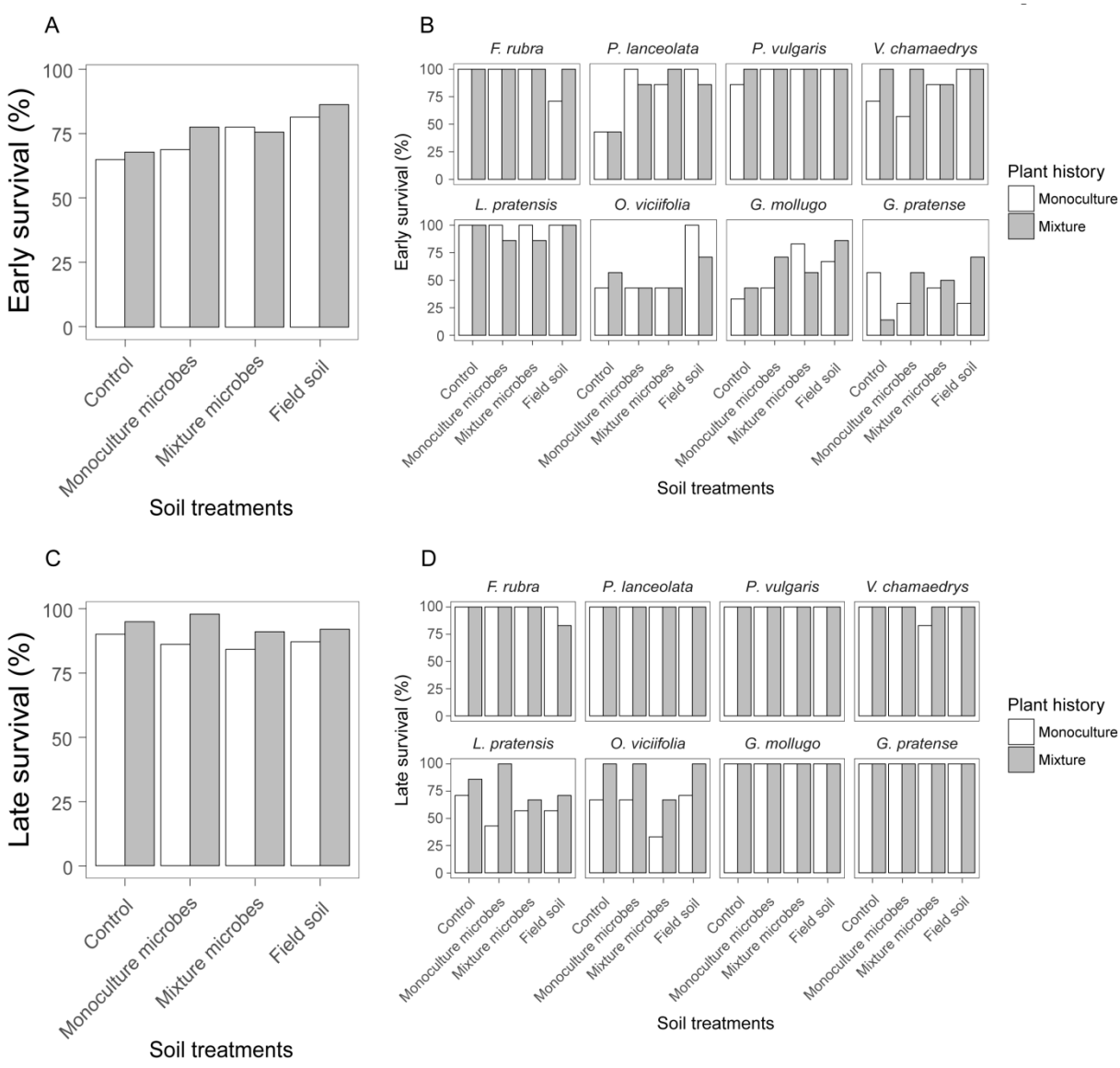


Figure 2. Survival of monoculture-type plants (white bars) and mixture-type plants (grey bars) in the four soil treatments: **A**, across all species until the first harvest; **B**, by species until the first harvest; **C**, across all species from the first harvest to the second harvest; **D**, by species from the first harvest to the second harvest. Bars in the figures A and B are proportion of survivors from all plants of the experiment. Bars in the figures C and D are proportion of plants that survived until the end of the experiment from plants that survived until the first harvest.

Table 3. Analysis of deviance (ANDEV) for plant survival before (early survival) and after (late survival) the first harvest.

	Early survival				Late survival			
Source of variation	Df	%-SS	*P*		Df	%-SS	*P*	
Block	6	2.4	0.008	**	6	4.0	<0.001	***
Table	12	2.9	0.048	*	12	5.9	<0.001	***
Functional group (FG)	3	12.3	<0.001	***	3	31.8	<0.001	***
Species within FG (SP)	4	9.2	<0.001	***	4	1.6	0.006	**
Plant history (PH)	1	0.3	0.147		1	3.1	<0.001	***
Soil treatment (ST)	3	2.8	<0.001	***	3	3.5	<0.001	***
Control vs. live soil treatments (C)	1	1.5	0.001	***	1	0.9	0.003	**
Field soil vs. monoculture or mixture microbes (F)	1	1.1	0.004	**	1	0.1	0.489	
Monoculture vs. mixture microbes (M)	1	0.2	0.254		1	2.5	<0.001	***
FG × PH	3	1.1	0.042	*	3	2.0	<0.001	***
FG × ST	9	4.1	<0.001	***	9	2.4	0.020	*
FG × C	3	1.8	0.005	**	3	0.1	0.792	
FG × F	3	2.2	0.001	**	3	2.1	<0.001	***
FG × M	3	0.1	0.805		3	0.2	0.579	
SP × PH	4	1.3	0.043	*	4	0.3	0.641	
SP × ST	12	2.7	0.071	.	12	0.6	0.969	
SP × C	4	1.4	0.037	*	4	0.2	0.754	
SP × F	4	1.1	0.097	.	4	0.2	0.692	
SP × M	4	0.2	0.835		4	0.1	0.889	
PH × ST	3	0.4	0.418		3	2.4	<0.001	***
PH × C	1	0.2	0.259		1	0.3	0.082	.
PH × F	1	0.0	0.954		1	0.5	0.030	*
PH × M	1	0.2	0.219		1	1.6	<0.001	***
Residuals	382	50.6			282	33.9		

Notes: Df, degrees of freedom; %-DVC, proportion of total deviance ; *P*, error probability

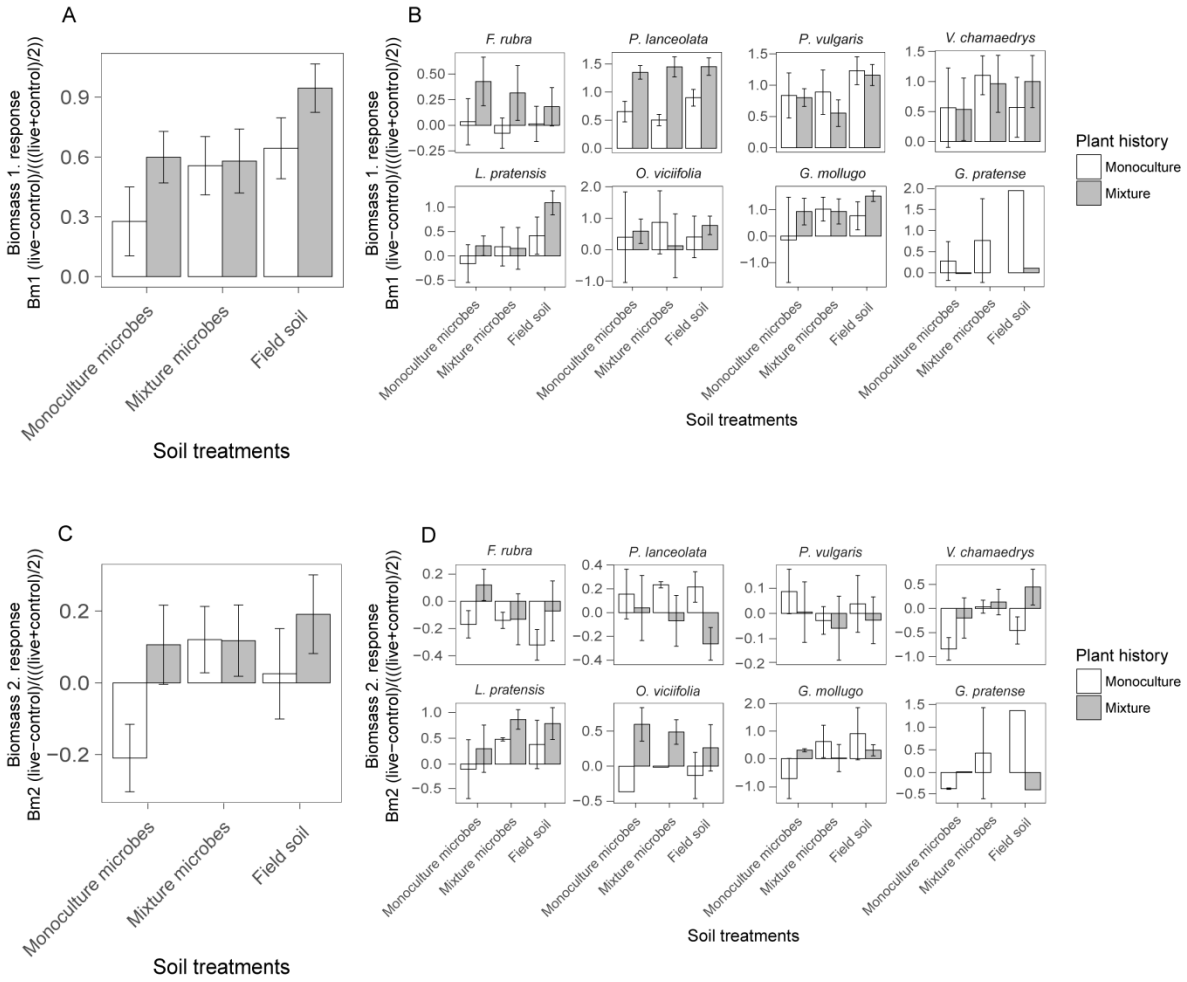


Figure 3. Biomass response ((Bm_{live} − $Bm_{control}$)/(((Bm_{live} + $Bm_{control}$)/2))) of monoculture-type plants (white bars) and mixture-type plants (grey bars) to the live soil treatments: **A**, across all species at the first harvest; **B**, by species at the first harvest, **C**, across all species at the second harvest; **D**, by species at the second harvest. Bars represent means ± standard errors.

Table 4. Analysis of variance (ANOVA) for plant biomass response at the first and second harvest.

	Biomass response Harvest 1.				Biomass response Harvest 2.			
Source of variation	Df	%-SS	*P*		Df	%-SS	*P*	
Intercept	1	45.4	<0.001	***	1	1.2	0.064	.
Block	6	4.3	0.070	.	6	11.5	<0.001	***
Table	12	5.6	0.220		11	4.1	0.409	
Functional group (FG)	3	12.0	<0.001	***	3	7.8	<0.001	***
Species (SP)	4	1.9	0.278		4	1.4	0.410	
Plant history (PH)	1	2.1	0.017	*	1	2.1	0.018	*
Soil treatment (ST)	2	2.4	0.036	*	2	0.7	0.383	
Field soil vs. monoculture or mixture microbes (F)	1	2.2	0.014	*	1	0.1	0.614	
Monoculture vs. mixture microbes (M)	1	0.2	0.414		1	0.6	0.198	
FG × PH	3	0.5	0.700		3	2.7	0.062	.
FG × ST	6	1.5	0.652		6	2.2	0.400	
FG × F	3	0.9	0.467		3	1.2	0.346	
FG × M	3	0.6	0.654		3	1.1	0.408	
SP × PH	4	3.7	0.038	*	4	4.4	0.020	*
SP × ST	8	2.1	0.663		8	4.6	0.125	
SP × F	4	1.3	0.470		4	0.9	0.661	
SP × M	4	0.8	0.683		4	3.8	0.037	*
PH × ST	2	0.6	0.411		2	2.0	0.063	.
PH × F	1	0.0	0.795		1	0.1	0.583	
PH × M	1	0.6	0.191		1	1.9	0.022	*
Residuals	157	56.5			131	46.9		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

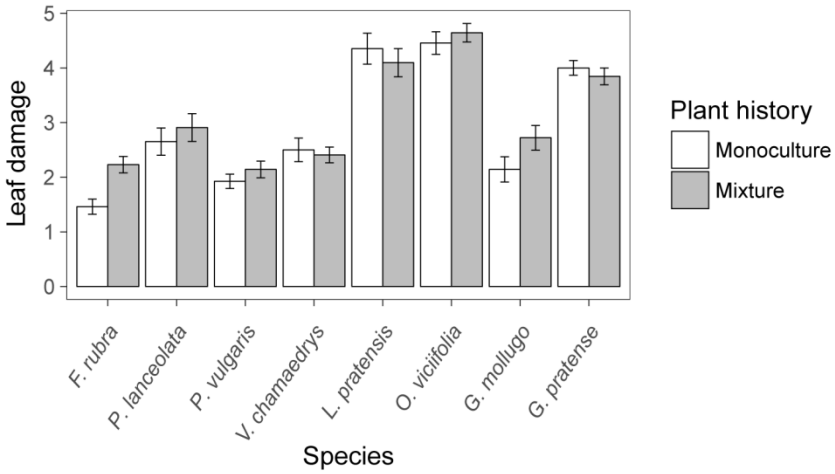


Figure 4. Amount of leaf damage (estimated from no damage (0) to strong damage (5)) of monoculture-type (white bars) and mixture-type plants (grey bars).

Table 5. ANOVA for plant leaf damage.

Source of variation	Df	%-SS	*P*	
Block	6	7.7	<0.001	***
Table	12	4.5	0.001	**
Functional group (FG)	3	39.6	<0.001	***
Species within FG (SP)	4	8.2	<0.001	***
Plant history (PH)	1	0.8	0.015	*
Soil treatment (ST)	3	0.1	0.917	
Control vs. live soil treatments (C)	1	0.0	0.692	
Field soil vs. monoculture or mixture microbes (F)	1	0.0	0.883	
Monoculture vs. mixture microbes (M)	1	0.0	0.566	
FG × PH	3	1.1	0.040	*
Residuals	286	38.0		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

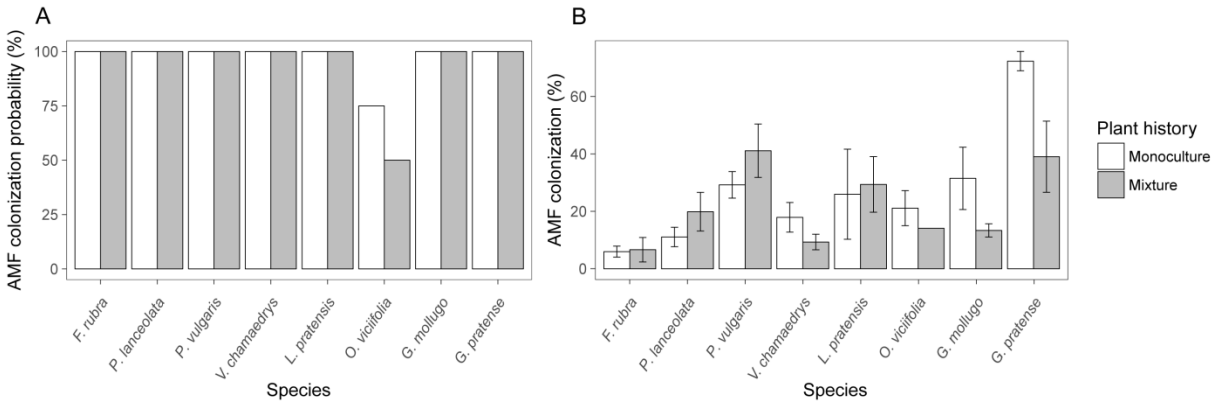


Figure 5. AMF colonization probability (A) and AMF colonization rate (B) in the roots of monoculture-type plants (white bars) and mixture-type plants (grey bars) grown in the field-soil treatment. The bars in figure A are proportions of colonized plants out of all plants that survived until the end of the experiment. Bars in figure B are means ± standard errors and only include plants with successful AMF-colonization (i.e. colonization rate > 0).

Table 6. ANOVA for AMF colonization rate of AMF colonized plants in the field-soil treatment.

Source of variation	Df	%-SS	*P*	
Block	6	12.2	0.015	*
Table	10	19.9	0.007	**
Functional group (FG)	3	8.9	0.009	**
Species within FG (SP)	4	17.5	<0.001	***
Plant history (PH)	1	0.3	0.488	
FG × PH	3	10.2	0.005	**
SP × PH	4	0.9	0.866	
Residuals	44	30.0		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

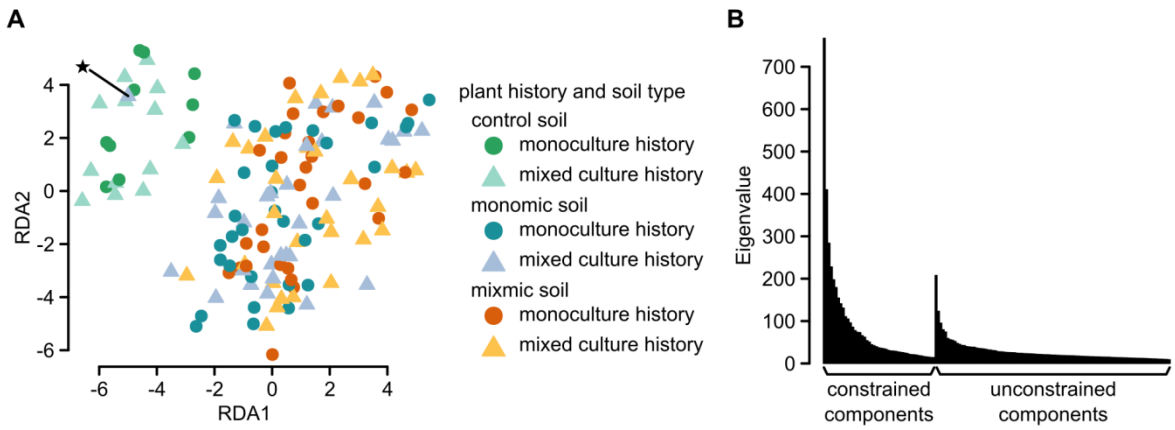


Figure 6. Redundancy analysis (RDA, Oksanen et al. 2017) using the normalized operational taxonomic unit (OTU) abundances of all samples sequenced. **A**, The two first components explained 17.4 % of the overall variance and separated the control soil from the microbial soils. The asterisk marks "Sample492" (*L. pratensis*, mixture-type plant, the soil treatment monoculture microbes), which clustered among the samples from the control soil. This sample was excluded as outlier from the analysis of differential OTU abundance. **B**, Eigenvalues of the constrained (RDA axes) and unconstrained (PCA axes) components identified in the RDA. The constrained components accounted for 60 % of the total variance.

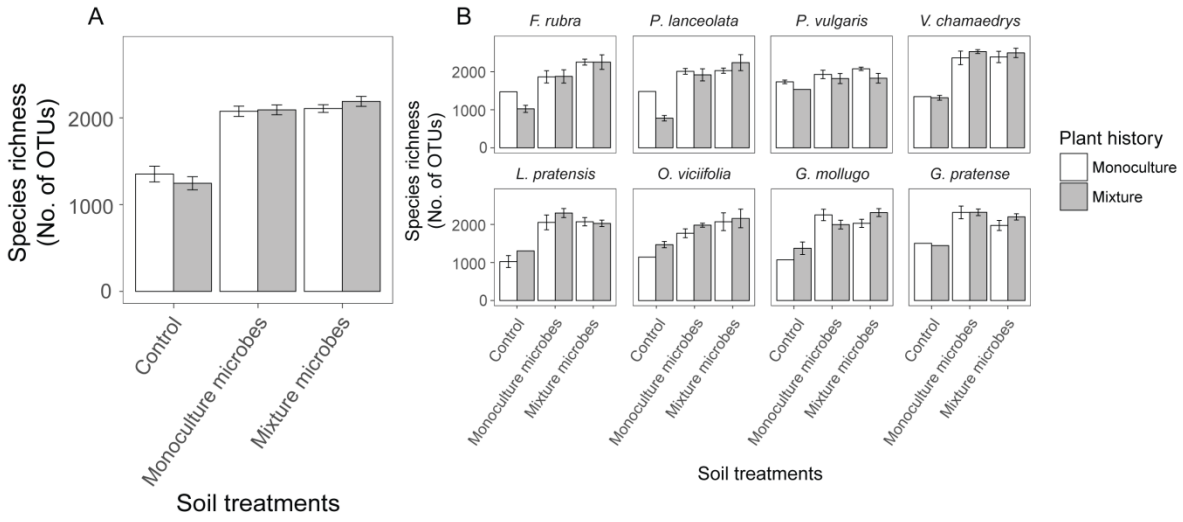


Figure 7. Bacterial species richness (number of OTUs) in the rhizosphere soil of monoculture-type plants (white bars) and mixture-type plants (grey bars): **A**, across plant species; **B**, separately for each plant species. Bars represent means ± standard errors.

Table 7. ANOVA for bacterial species (number of OTUs) in the rhizosphere soil of plants.

Source of variation	Df	%-SS	*P*	
Species (SP)	7	6.4	<0.001	***
Plant history (PH)	1	0.0	0.908	
Soil treatment (ST)	2	30.5	<0.001	***
Control vs. microbial soil treatments (C)	1	30.2	<0.001	***
Monoculture vs. mixture microbes (M)	1	0.3	0.173	
SP × PH	7	1.4	0.287	
SP × ST	14	5.4	0.005	**
SP × C	7	2.9	0.014	*
SP × M	7	2.5	0.035	*
PH × ST	2	0.2	0.499	
PH × C	1	0.1	0.367	
PH × M	1	0.1	0.448	
SP × PH × ST	14	2.4	0.381	
SP × PH × C	7	1.2	0.370	
SP × PH × M	7	1.2	0.388	
Residuals	98	15.2		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

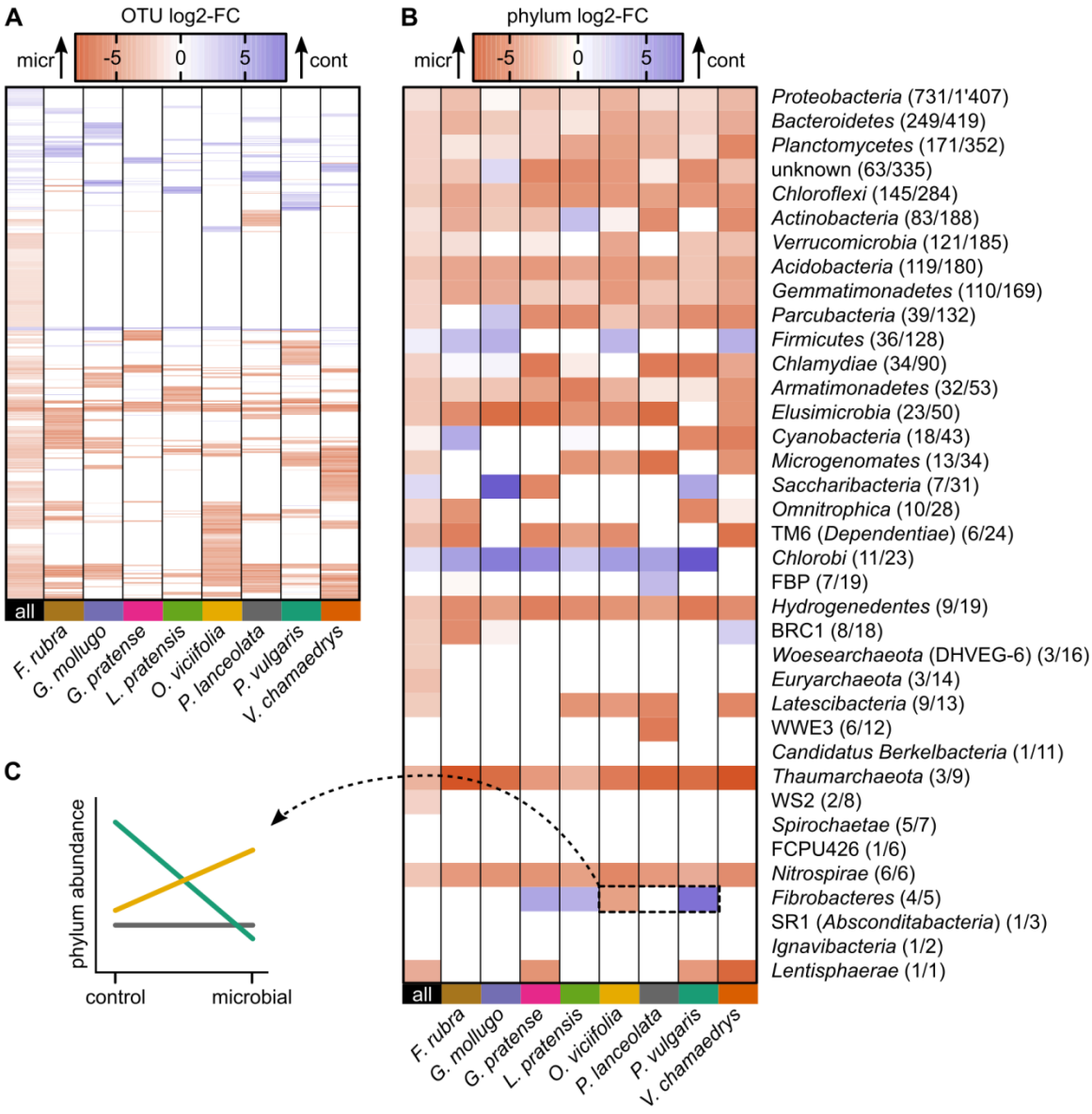


Figure 8.OTUs with significant differences in abundance between the control and the microbial soil treatments across all plant species or for each plant species separately (contrast 2. in Table 8). **A**, Differences in abundance of the significant OTUs; **B**, Differences in abundance of the different microbial phyla (average of the significant OTUs assigned to a phylum). Numbers in parenthesis indicate the number of OTUs significant in any of the contrasts listed in Table 8 (2'091 OTUs) and the total number of OTUs within the entire data set (4'339 OTUs) assigned to a phylum. **A**,**B** Red and blue for higher OTU/phylum abundance in the microbial soil treatments and the control soil, respectively. White for insignificant differences. **C**, The drawing illustrates how interactions between the plant species and the soil contrast can be inferred from the heatmap. Abbreviations: log2-FC, log2 fold-change; cont, control soil; micr, microbial soil treatments.

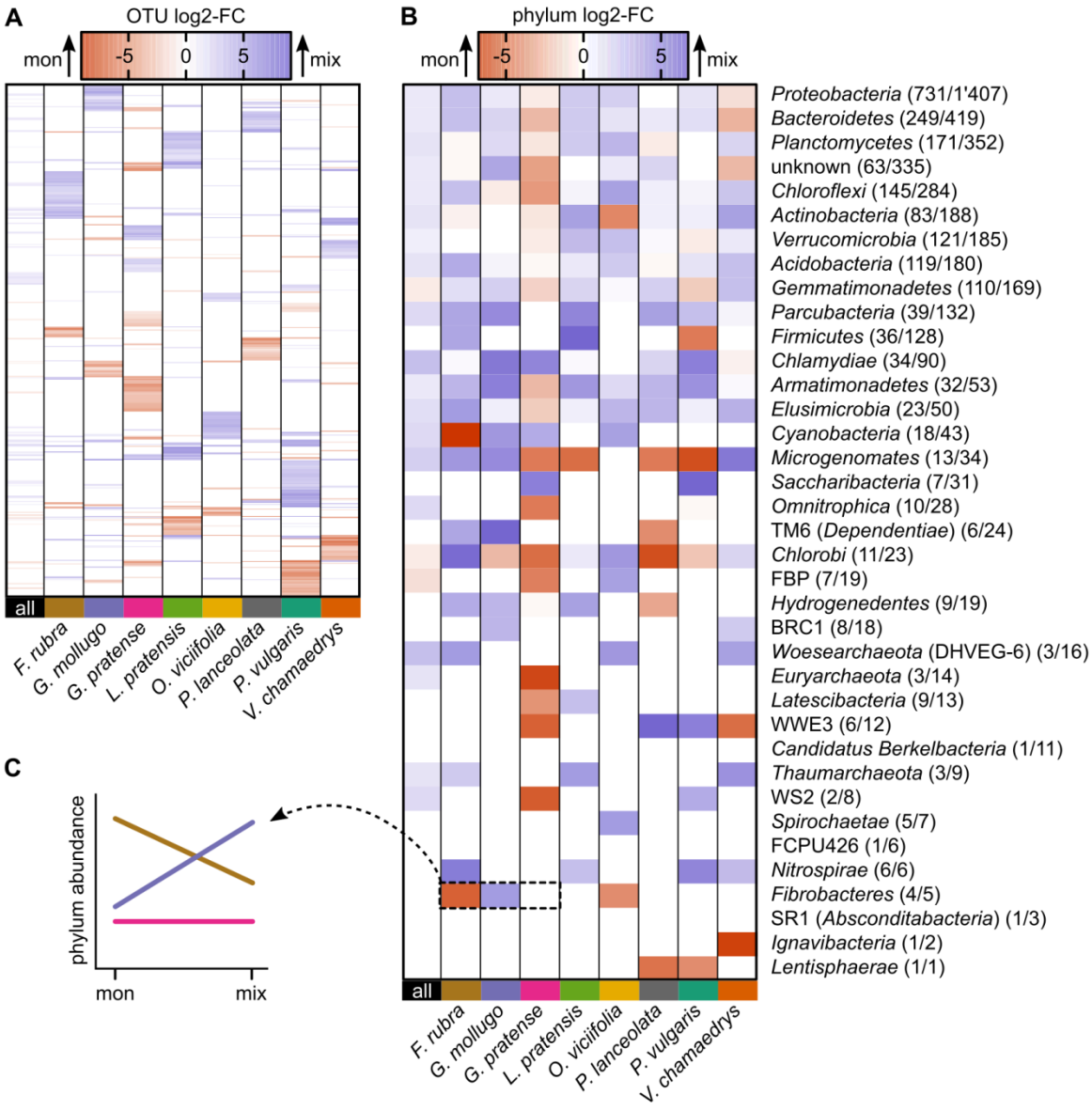


Figure 9. OTUs with significant differences in abundance between the two microbial soil treatments across all plant species or for each plant species separately (contrast 3. in Table 8). **A**, Differences in abundance of the significant OTUs; **B**, Differences in abundance of the different microbial phyla (average of the significant OTUs assigned to a phylum). Numbers in parenthesis indicate the number of OTUs significant in any of the contrasts listed in Table 8 (2'091 OTUs) and the total number of OTUs within the entire data set (4'339 OTUs) assigned to a phylum. **A**, **B**, Red and blue for higher OTU/phylum abundance in monoculture microbes and mixture microbes, respectively. White for insignificant differences. **C**, The drawing illustrates how interactions between the plant species and the soil contrast can be inferred from the heatmap. Abbreviations: log2-FC, log2 fold-change; mon, monoculture microbes; mix, mixture microbes.

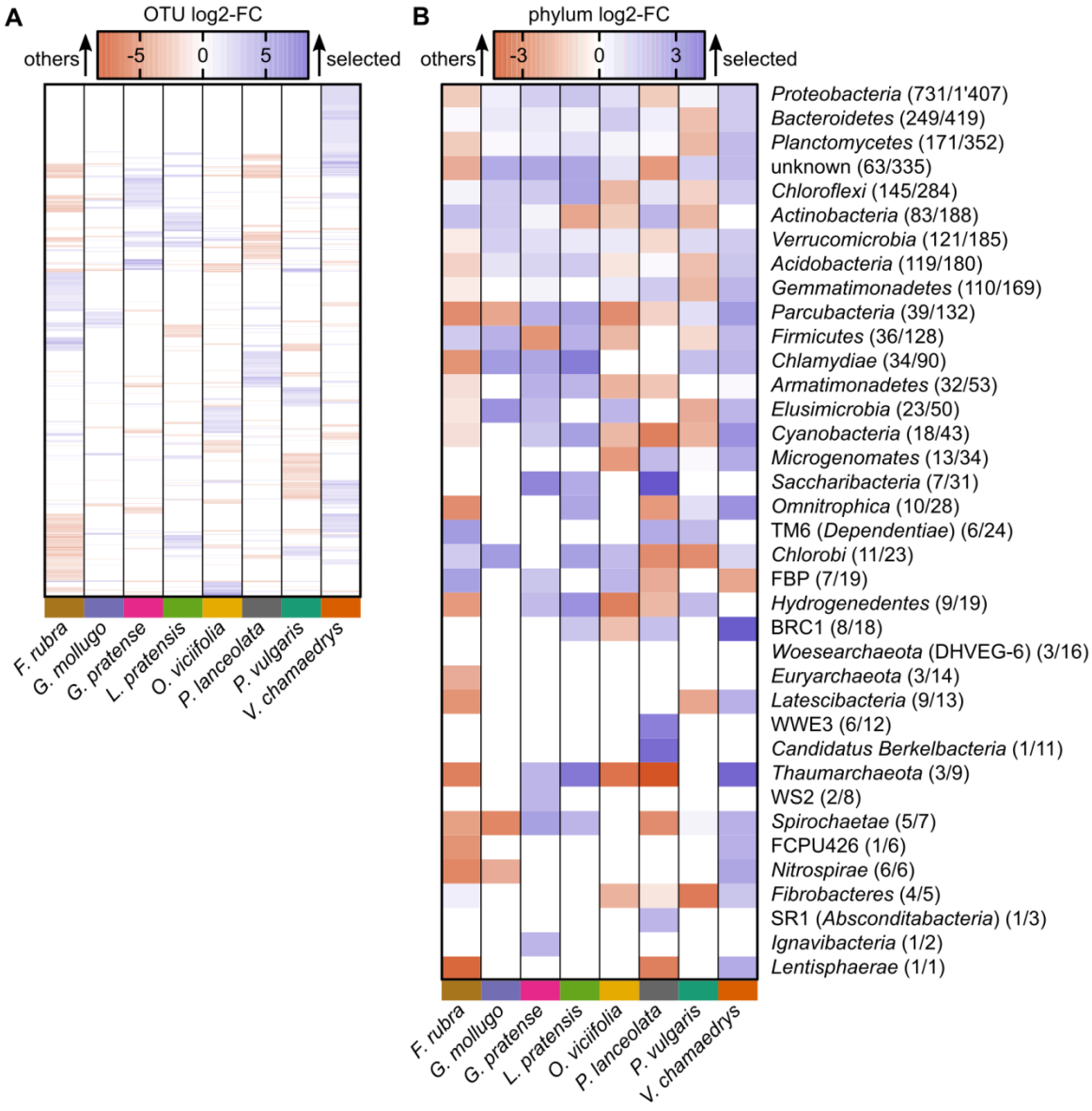


Figure 10. OTUs with significant differences in abundance between one plant species compared to all other plant species (contrast 4. in Table 8). **A**, Differences in abundance of the significant OTUs; **B**, Differences in abundance of the different microbial phyla (average of the significant OTUs assigned to a phylum). Numbers in parenthesis indicate the number of OTUs significant in any of the contrasts listed in Table 8 (2'091 OTUs) and the total number of OTUs within the entire data set (4'339 OTUs) assigned to a phylum. **A** ,**B**, Blue and red for higher OTU/phylum abundance in the specific plant species ("selected") and all other plant species ("others"), respectively. White for insignificant differences. Abbreviations: log2-FC, log2 fold-change.

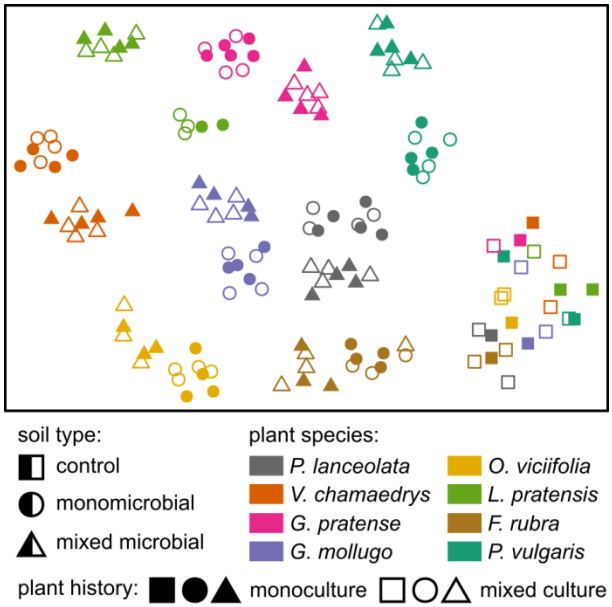


Figure 11. t-SNE map (Maaten & Hinton, 2008)of all samples sequenced and analyzed (excluding the outlier "Sample492"). The map was generated using normalized abundances of OTUs identified as significantly differentially abundant within any of the contrasts tested in this study (2'091 OTUs, Table 8). Note that t-SNE projection axes are arbitrary and dimensions are therefore not shown.

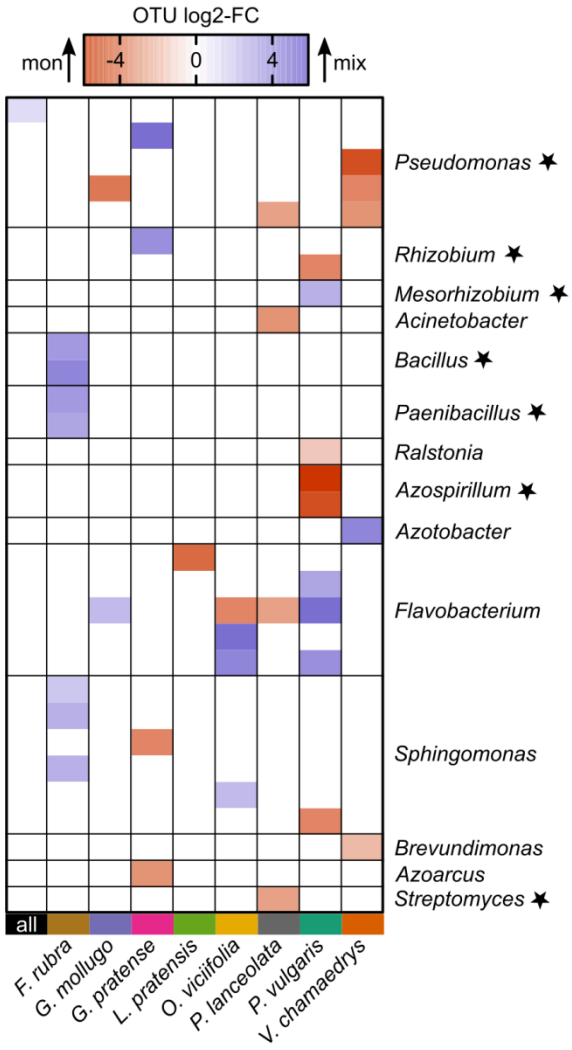


Figure 12. OTUs assigned to taxa comprising plant growth promoting microbes (Ahemad & Kibret, 2014) with significant differences in abundance between the two microbial soil treatments across all plant species or for each plant species separately (each row corresponds to one OTU). Red and blue for higher OTU abundance in monoculture microbes and mixture microbes, respectively. White for insignificant differences. Taxa marked with an asterisk comprise species known to promote plant growth indirectly by antagonizing plant pathogens (Ahemad & Kibret, 2014). Abbreviations: log2-FC, log2 fold-change.

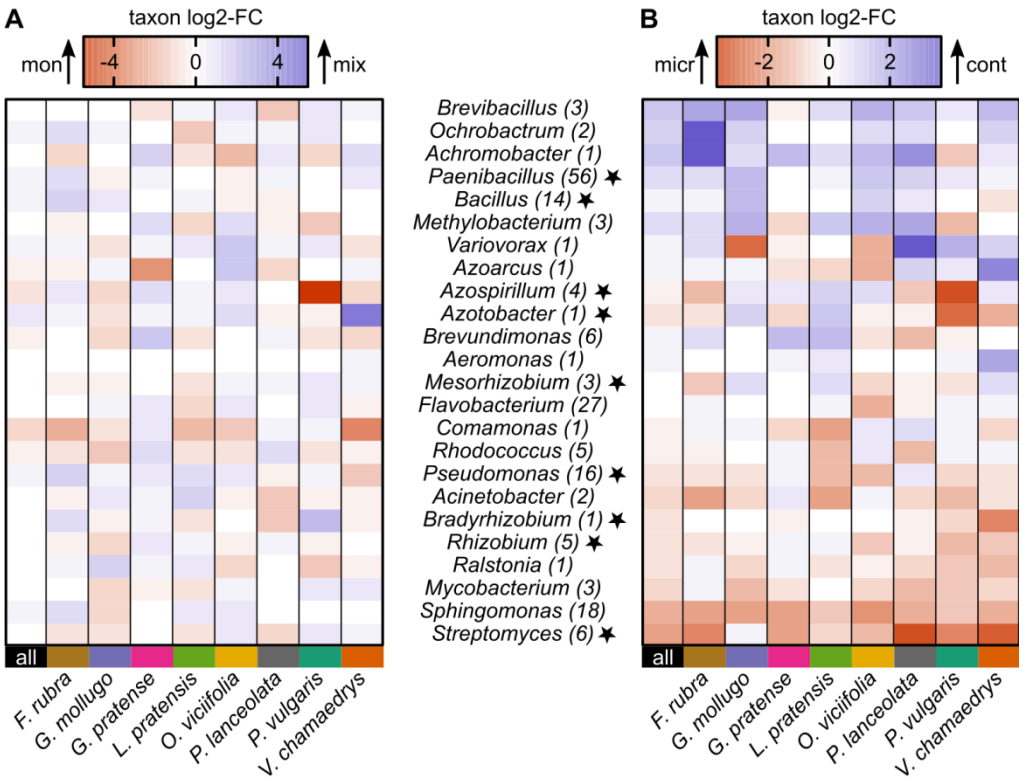


Figure 13. Differential abundance of taxa comprising plant growth promoting microbes (Ahemad & Kibret, 2014) (average difference across all OTUs assigned to a taxon, irrespective of their individual significance). **A**, Comparison between the two microbial soil treatments. Red and blue for higher taxon abundance in monoculture microbes and mixture microbes, respectively. **B**, As a reference, comparison between the control soil ("cont") and the microbial soil treatments ("micr"). Red and blue for higher taxon abundance in the microbial soil treatments and the control soil, respectively. Numbers in parentheses indicate the number of OTUs assigned to a taxon. Taxa marked with an asterisk comprise species known to promote plant growth indirectly by antagonizing plant pathogens (Ahemad & Kibret, 2014). Abbreviations: log2-FC, log2 fold-change.

Table 8. The number of operational taxonomic units (OTUs) exhibiting significant differential abundance in any of the contrasts tested in this study (FDR <= 0.01 and abs(logFC) >= 1). Numbers in parentheses refer to the number of OTUs exhibiting higher abundance in the first and the second group of the contrast, respectively. Contrasts included either all species or each species individually (columns). The contrast comparing monoculture- and mixture-type plants ("PH: mix *vs.* mono") was tested across the entire data set and within the specific soil treatments. Considering that plant history had a weak effect on the composition of the microbiomes, the other contrasts comparing the different soil treatments ("SO: control *vs.* others" and "SO: mixmic *vs.* monomic") were only tested across the entire data set and within the individual plant species. Likewise, the contrast comparing one specific plant species to all others ("SP: one *vs.* others") was only tested across the entire data set and within the specific soil treatments. It is important to note that there were only three samples from the control soil per species (i.e., one with monoculture and two with mixed culture plant history or *vice versa*). The contrast **1. a)** was therefore only tested across all species. Abbreviations: PH, plant history (mono and mix for monoculture and mixed culture history, respectively); SO, soil treatment (monomic and mixmic for monoculture microbes and mixture microbes, respectively); SP, plant species; FesRub, *F. rubra*; GalMol, *G. mollugo*; GerPra, *G. pratense*; LatPra, *L. pratensis*; OnoVic, *O. viciifolia*; PlaLan, *P. lanceolata*; PruVul, *P. vulgaris*; VerCha, *V. chamaedrys*.

Contrast / Species	All	FesRub	GalMol	GerPra	LatPra	OnoVic	PlaLan	PruVul	VerCha
1. PH: mix *vs.* mono	1 (0/1)	5 (4/1)	6 (5/1)	0	3 (1/2)	1 (1/0)	6 (6/0)	11 (4/7)	1 (0/1)
a) within control SO	12 (6/6)	-	-	-	-	-	-	-	-
b) within monomic SO	0	0	2 (2/0)	0	2 (1/1)	0	0	5 (0/5)	0
c) within mixmic SO	0	0	0	0	0	0	1 (1/0)	2 (1/1)	0
2. SO: control *vs.* others	972 (156/816)	321 (76/245)	267 (92/175)	136 (29/107)	171 (46/125)	339 (37/302)	295 (70/225)	296 (77/219)	366 (53/313)
3. SO: mixmic *vs.* monomic	137 (105/32)	136 (108/28)	135 (85/50)	212 (68/144)	154 (112/42)	103 (77/26)	126 (69/57)	241 (144/97)	133 (68/65)
4. SP: one *vs.* others	-	498 (192/306)	129 (84/45)	236 (167/69)	221 (162/59)	264 (148/116)	290 (133/157)	299 (118/181)	500 (422/78)
a) within control SO	-	96 (61/35)	40 (35/5)	54 (50/4)	56 (54/2)	35 (24/11)	69 (42/27)	63 (49/14)	77 (69/8)
b) within monomic SO	-	463 (182/281)	218 (146/72)	423 (290/133)	157 (101/56)	325 (194/131)	302 (178/124)	346 (173/173)	593 (497/96)
c) within mixmic SO	-	346 (178/168)	223 (130/93)	286 (142/144)	393 (285/108)	281 (208/73)	337 (163/174)	402 (198/204)	454 (393/61)

SUPPORTING INFORMATION

Table S1. The composition of plant functional groups and species in the eight species mixtures plots of the Jena Experiment from which mixture-type plants and their rhizosphere soil samples were collected. Plot code refers the plot in the Jena Experiment. The columns of each eight species represent plant functional groups and species present (1) and not present (0) in the plot.

Functional group	Species	*F. rubra*	*G. mollugo*	*G. pratense*	*L. pratensis*	*O. vicilfolia*	*P. lanceolata*	*P. vulgaris*	*V. chamaedrys*
Small herb	*Ajuga reptans*	1	0	0	0	0	0	0	0
Grass	*Anthoxanthum odoratum*	1	0	0	0	0	0	1	0
Tall herb	*Anthriscus sylvestris*	0	0	1	0	0	1	0	0
Grass	*Avenula pubescens*	1	0	0	0	0	0	0	0
Grass	*Bromus hordeaceus*	1	0	0	0	0	0	0	0
Tall herb	*Crepis biennis*	0	1	0	0	1	0	0	0
Grass	*Cynosurus cristatus*	0	0	0	0	0	0	0	1
Tall herb	*Daucus carota*	0	0	0	0	0	1	0	0
Grass	***F. rubra***	1	0	0	0	0	0	0	0
Tall herb	***G. mollugo***	0	1	1	0	1	0	0	0
Tall herb	***G. pratense***	0	0	1	0	0	0	0	0
Small herb	*Glechoma hederacea*	0	0	0	1	0	0	0	1
Tall herb	*Heracleum sphondylium*	0	0	1	0	0	0	0	0
Tall herb	*Knautia arvensis*	0	0	1	0	0	0	1	0
Legume	***L. pratensis***	0	0	0	1	0	0	0	0
Small herb	*Leontodon autumnalis*	0	0	0	1	0	0	0	0
Small herb	*Leontodon hispidus*	0	1	0	0	1	1	0	0
Tall herb	*Leucanthemum vulgare*	0	0	1	0	0	0	0	0
Legume	*Lotus corniculatus*	0	1	0	0	1	0	0	1
Grass	*Luzula campestris*	0	0	0	0	0	1	0	0
Legume	*Medicago lupulina*	0	1	0	0	1	0	0	1
Legume	***O. viciifolia***	0	1	0	0	1	0	0	0
Grass	*Phleum pratense*	0	0	0	0	0	0	0	1
Small herb	***P. lanceolata***	1	0	0	0	0	1	0	0
Small herb	*Plantago media*	0	1	0	1	1	0	0	0
Small herb	*Primula veris*	0	0	0	0	0	0	0	1
Small herb	***P. vulgaris***	0	0	0	0	0	0	1	0
Tall herb	*Ranunculus acris*	0	0	1	0	0	0	0	0
Tall herb	*Sanguisorba officinalis*	0	1	1	0	1	0	0	0
Small herb	*Taraxacum officinale*	1	0	0	1	0	0	0	0
Legume	*Trifolium campestre*	0	0	0	1	0	1	0	0
Grass	*Trisetum flavescens*	0	0	0	0	0	1	0	1
Legume	*Trifolium fragiferum*	0	0	0	1	0	1	0	0
Legume	*Trifolium pratense*	0	0	0	0	0	0	1	0
Small herb	***V. chamaedrys***	1	0	0	0	0	0	0	1
Legume	*Vicia cracca*	0	0	0	1	0	0	0	0
	Plot code:	B4A08	B2A21	B2A12	B2A17	B2A21	B1A14	B2A01	B1A03

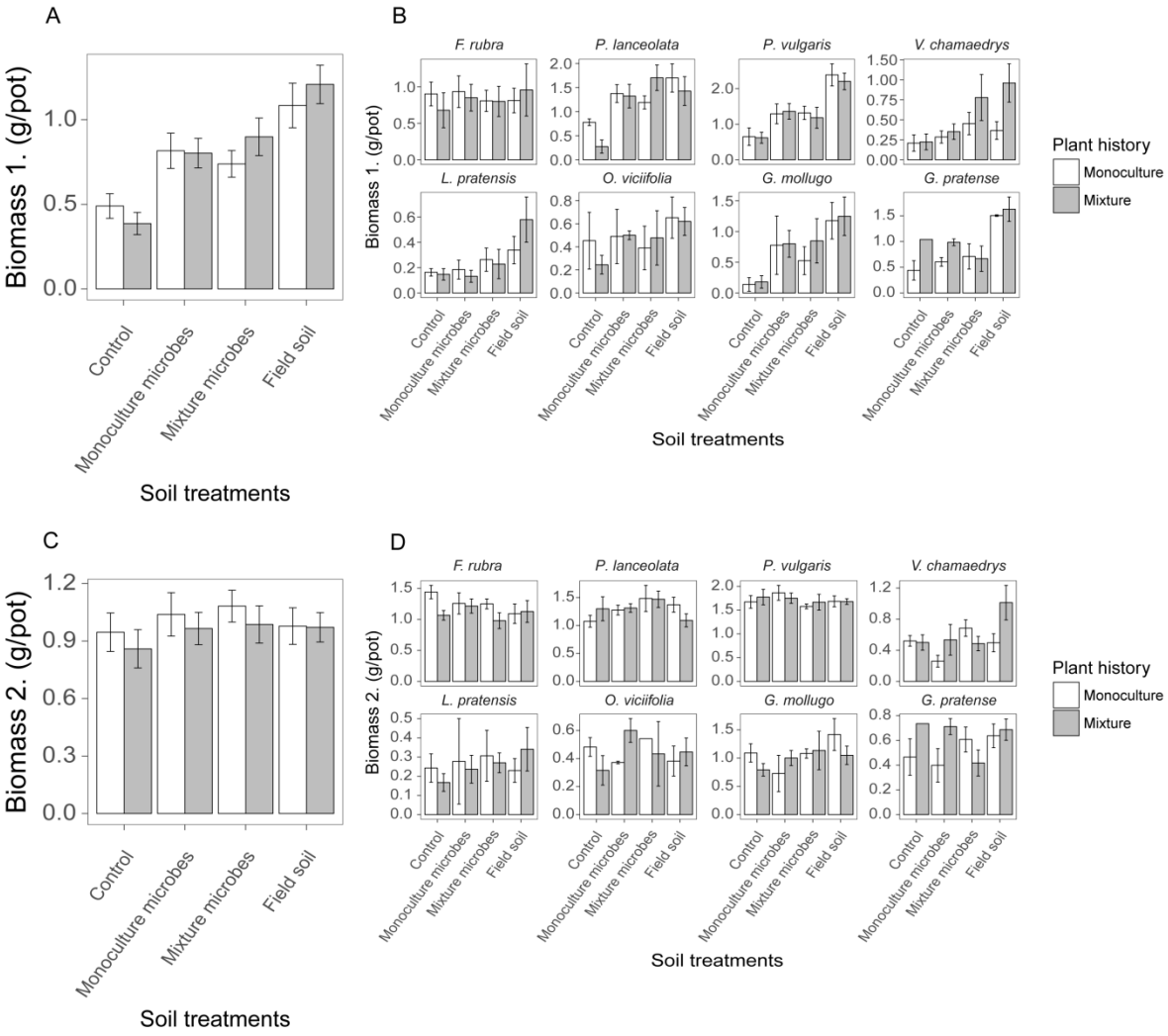


Figure S1. Biomass production of monoculture-type plants (white bars) and mixture-type plants (grey bars): **A**, across all species at the first harvest; **B**, by species at the first harvest, **C**, across all species at the second harvest; **D**, by species at the second harvest. Bars represent means ± standard errors.

Table S2. ANOVA for plant biomass production at the first and second harvest.

	Bm1 (Harvest 1.)			Bm2 (Harvest 2.)		
Source of variation	Df	%-SS	*P*	Df	%-SS	*P*
Block	6	11.0	<0.001 ***	6	4.6	<0.001 ***
Table	12	3.7	<0.001 ***	12	5.0	<0.001 ***
Functional group (FG)	3	12.1	<0.001 ***	3	33.5	<0.001 ***
Species (SP)	4	13.8	<0.001 ***	4	31.8	<0.001 ***
Plant history (PH)	1	0.2	0.193	1	0.0	0.576
Soil treatment (ST)	3	9.8	<0.001 ***	3	0.1	0.613
Control vs. live soil treatments (C)	1	5.6	<0.001 ***	1	0.1	0.321
Field soil vs. monoculture or mixture microbes (F)	1	4.2	<0.001 ***	1	0.1	0.365
Monoculture vs. mixture microbes (M)	1	0.0	0.795	1	0.0	0.998
FG × PH	3	0.3	0.306	3	0.4	0.122
FG × ST	9	3.6	<0.001 ***	9	0.7	0.370
FG × C	3	2.5	<0.001 ***	3	0.2	0.364
FG × F	3	1.0	0.014 *	3	0.3	0.295
FG × M	3	0.2	0.639	3	0.2	0.411
SP × PH	4	0.6	0.144	4	0.4	0.224
SP × ST	12	3.0	0.001 **	12	1.4	0.114
SP × C	4	1.0	0.021 *	4	0.1	0.898
SP × F	4	1.8	0.001 ***	4	0.8	0.032 *
SP × M	4	0.2	0.668	4	0.5	0.166
PH × ST	3	0.2	0.484	3	0.3	0.293
PH × C	1	0.2	0.201	1	0.2	0.155
PH × F	1	0.0	0.932	1	0.0	0.800
PH × M	1	0.1	0.369	1	0.1	0.201
Residuals	282	25.0		256	19.1	

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

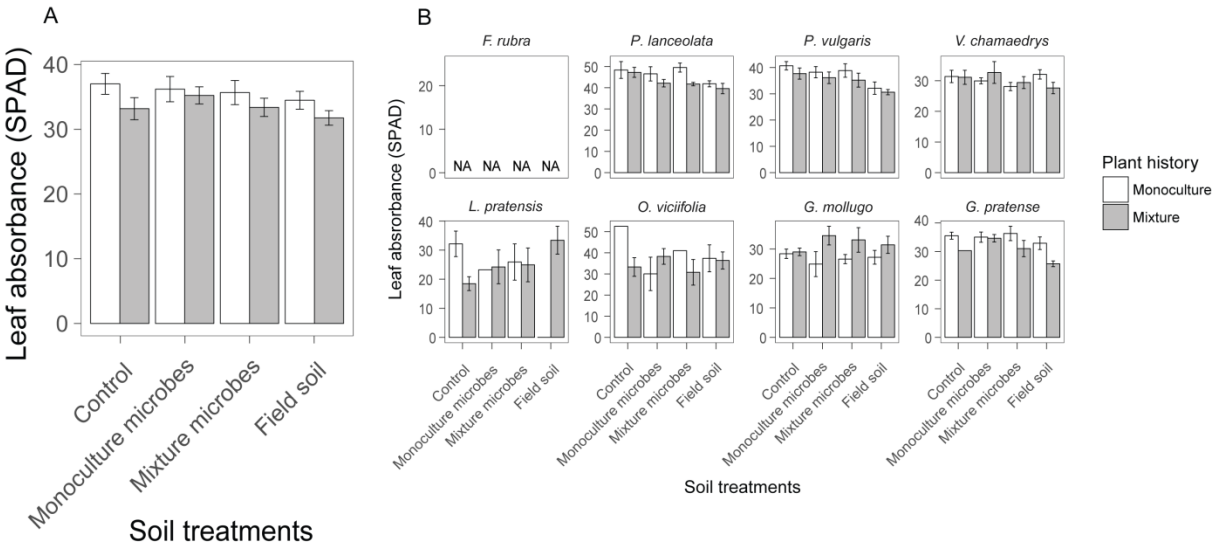


Figure S2. Leaf absorbance (SPAD) of monoculture-type plants (white bars) and mixture-type plants (grey bars) **A**, across all species; **B**, by species. Bars are means ± standard errors.

Table S3. ANOVA for plant leaf absorbance.

Source of variation	Df	%-SS	*P*	
Block	6	2.0	0.117	
Table	12	6.6	0.001	**
Functional group (FG)	3	9.6	<0.001	***
Species within FG (SP)	4	25.1	<0.001	***
Plant history (PH)	1	0.8	0.045	*
Soil treatment (ST)	3	2.2	0.010	**
Control vs. live soil treatments (C)	1	0.7	0.063	.
Field soil vs. monoculture or mixture microbes (F)	1	1.2	0.012	*
Monoculture vs. mixture microbes (M)	1	0.4	0.172	
FG × PH	2	0.7	0.168	
FG × ST	6	2.1	0.093	.
FG × C	2	0.3	0.422	
FG × F	2	1.7	0.013	*
FG × M	2	0.1	0.817	
SP × PH	4	1.9	0.047	*
SP × ST	12	1.9	0.626	
SP × C	4	0.5	0.636	
SP × F	4	1.1	0.203	
SP × M	4	0.3	0.857	
PH × ST	3	1.1	0.123	
PH × C	1	0.5	0.108	
PH × F	1	0.3	0.252	
PH × M	1	0.4	0.166	
FG × PH × ST	6	2.2	0.081	.
FG × PH × C	2	1.9	0.007	**
FG × PH × F	2	0.1	0.806	
FG × PH × M	2	0.2	0.640	
Residuals	183	34.6		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

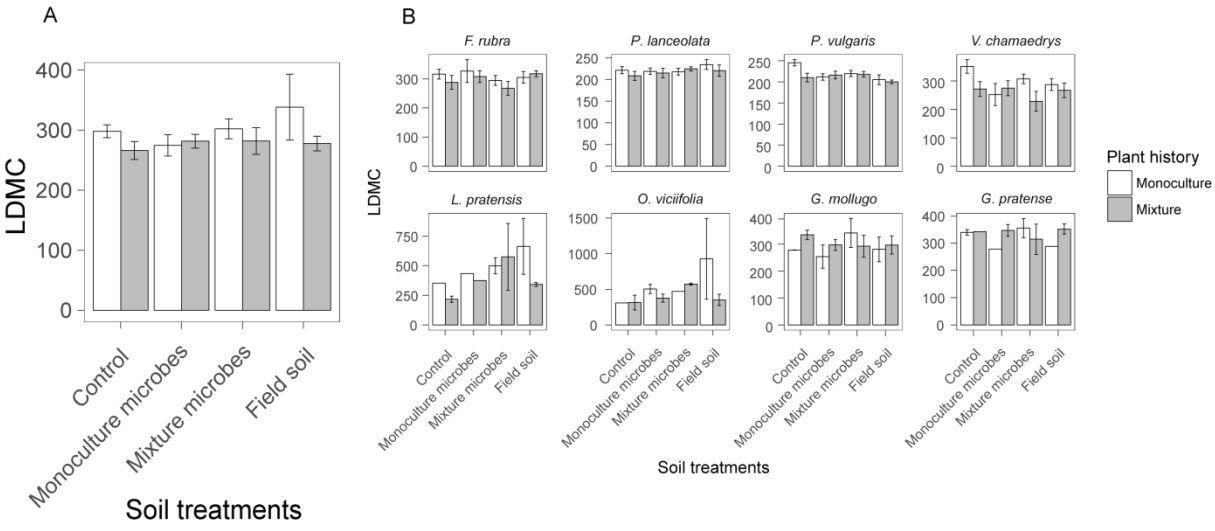


Figure S3. Leaf dry matter content of monoculture-type plants (white bars) and mixture-type plants (grey bars) **A**, across all species; **B**, by species. Bars are means ± standard errors.

Table S4. ANOVA for plant LDMC.

Source of variation	Df	%-SS	*P*	
Block	6	5.6	<0.001	***
Table	12	2.7	0.314	
Functional group (FG)	3	19.5	<0.001	***
Species within FG (SP)	4	1.7	0.074	.
Plant history (PH)	1	0.8	0.042	*
Soil treatment (ST)	3	0.5	0.438	
Control vs. live soil treatments (C)	1	0.3	0.206	
Field soil vs. monoculture or mixture microbes (F)	1	0.2	0.307	
Monoculture vs. mixture microbes (M)	1	0.0	0.795	
FG × PH	3	3.8	<0.001	***
FG × ST	9	5.5	0.002	**
FG × C	3	3.6	0.001	***
FG × F	3	0.9	0.203	
FG × M	3	1.0	0.176	
SP × PH	4	0.6	0.565	
SP × ST	12	0.5	0.998	
SP × C	4	0.1	0.949	
SP × F	4	0.3	0.814	
SP × M	4	0.0	0.996	
PH × ST	3	0.5	0.484	
PH × C	1	0.0	0.981	
PH × F	1	0.4	0.135	
PH × M	1	0.0	0.650	
FG × PH × ST	9	5.1	0.003	**
FG × PH × C	3	0.3	0.676	
FG × PH × F	3	4.3	<0.001	***
FG × PH × M	3	0.5	0.473	
Residuals	210	41.1		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

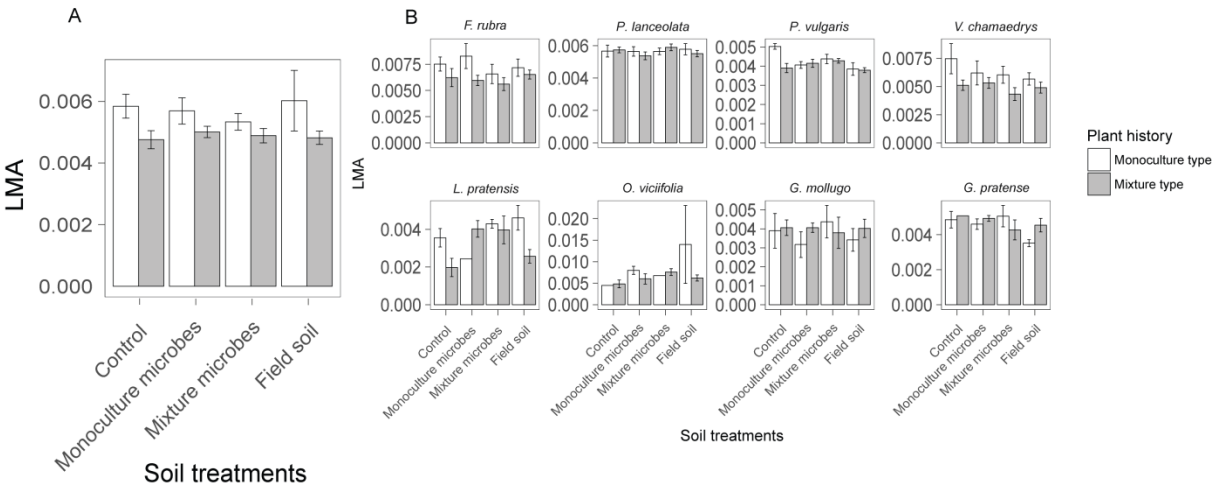


Figure S4. Leaf mass per area (LMA) of monoculture-type plants (white bars) and mixture-type plants (grey bars) **A**, across all species; **B**, by species. Bars are means ± standard errors.

Table S5. ANOVA for plant LMA.

Source of variation	Df	%-SS	*P*	
Block	6	10.1	<0.001	***
Table	12	1.3	0.942	
Functional group (FG)	3	8.8	<0.001	***
Species within FG (SP)	4	9.3	<0.001	***
Plant history (PH)	1	1.9	0.005	**
Soil treatment (ST)	3	0.1	0.920	
Control vs. live soil treatments (C)	1	0.0	0.750	
Field soil vs. monoculture or mixture microbes (F)	1	0.1	0.580	
Monoculture vs. mixture microbes (M)	1	0.0	0.769	
FG × PH	3	2.8	0.011	*
Residuals	267	65.4		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

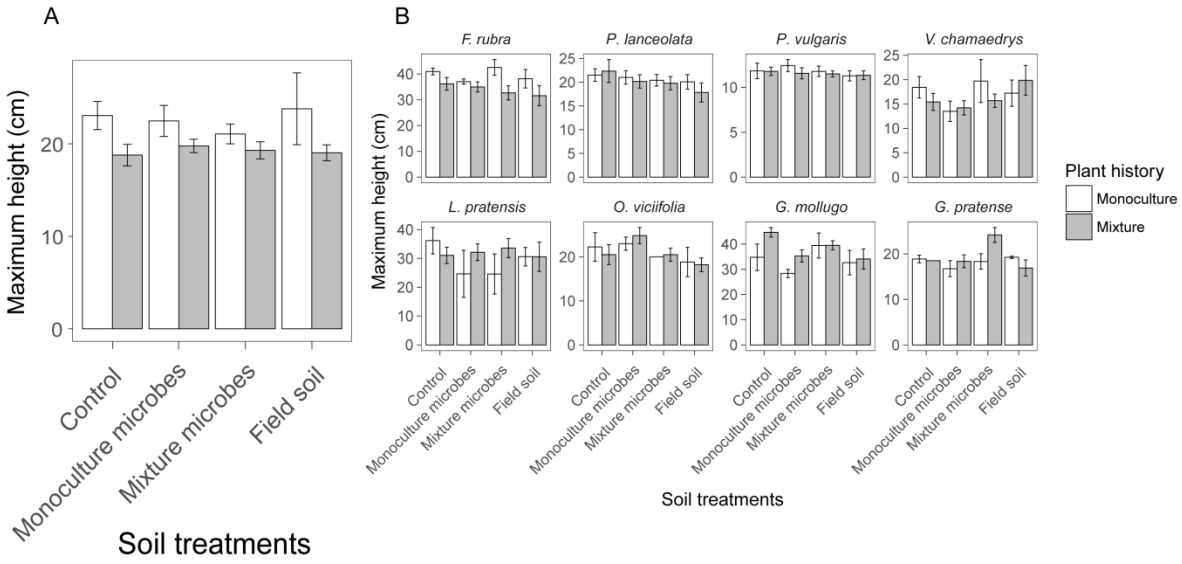


Figure S5. Maximum height of monoculture-type plants (white bars) and mixture-type plants (grey bars) **A**, across all species; **B**, by species. Bars are means ± standard errors.

Table S6. ANOVA for plant maximum height.

Source of variation	Df	%-SS	*P*	
Block	6	4.0	<0.001	***
Table	12	3.0	<0.001	***
Functional group (FG)	3	51.3	<0.001	***
Species within FG (SP)	4	17.1	<0.001	***
Plant history (PH)	1	0.1	0.228	
Soil treatment (ST)	3	0.5	0.089	.
Control vs. live soil treatments (C)	1	0.4	0.030	*
Field soil vs. monoculture or mixture microbes (F)	1	0.1	0.404	
Monoculture vs. mixture microbes (M)	1	0.1	0.297	
FG × PH	3	1.2	0.001	**
FG × ST	9	0.9	0.255	
FG × C	3	0.1	0.826	
FG × F	3	0.2	0.529	
FG × M	3	0.6	0.043	*
SP × PH	4	0.1	0.855	
Residuals	273	20.5		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

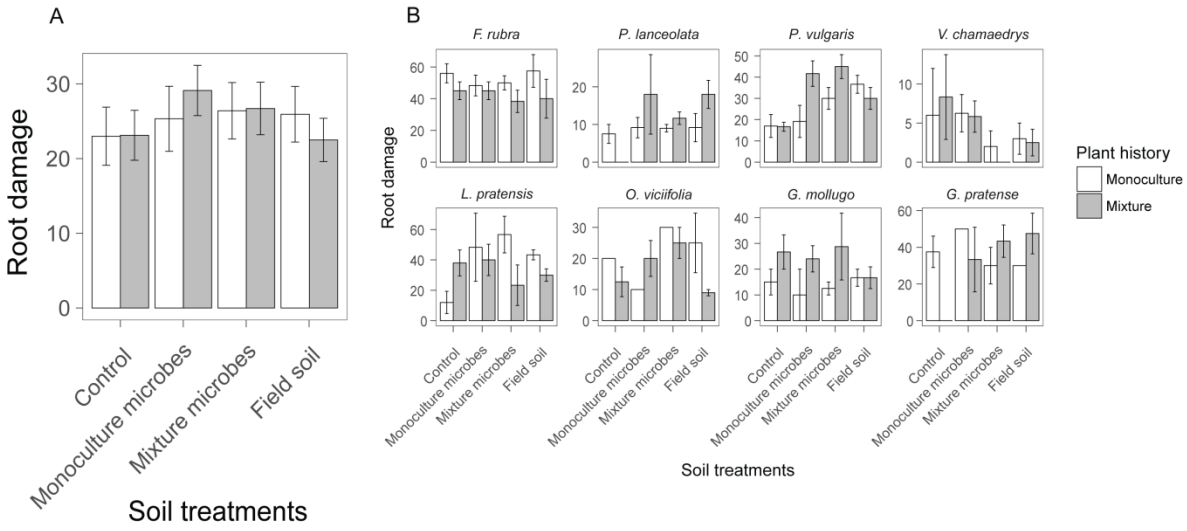


Figure S6. Root damage of monoculture-type plants (white bars) and mixture-type plants (grey bars) **A**, across all species; **B**, by species. Bars are means ± standard errors.

Table S7. ANOVA for plant root damage.

Source of variation	Df	%-SS	*P*	
Block	5	1.3	0.223	
Table	10	1.6	0.546	
Functional group (FG)	3	27.1	<0.001	***
Species within FG (SP)	4	22.0	<0.001	***
Plant history (PH)	1	0.0	0.729	
Soil treatment (ST)	3	1.3	0.079	.
Control vs. live soil treatments (C)	1	1.2	0.009	**
Field soil vs. monoculture or mixture microbes (F)	1	0.0	0.926	
Monoculture vs. mixture microbes (M)	1	0.0	0.866	
FG × PH	3	2.0	0.012	*
Residuals	240	43.5		

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability

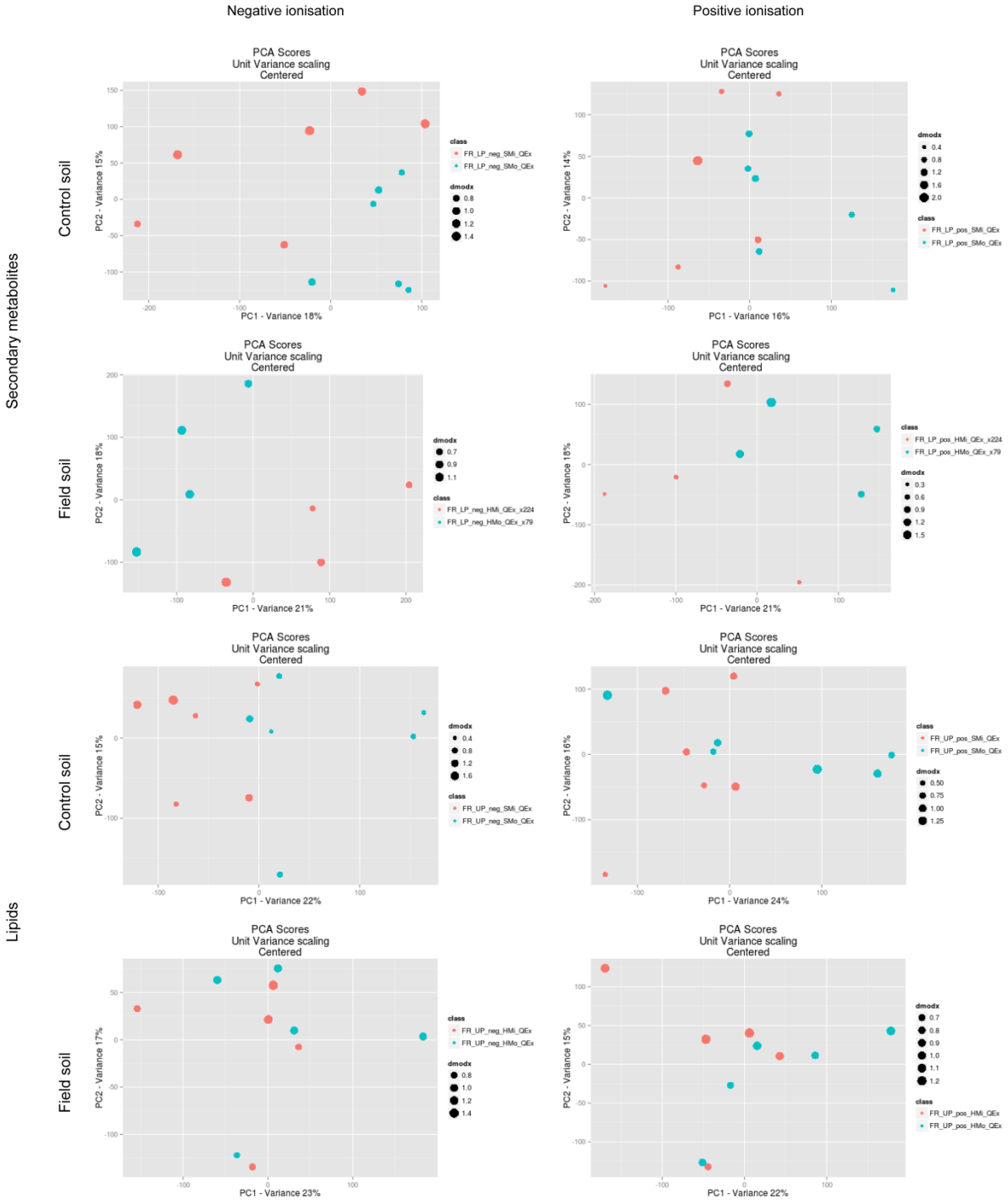


Figure S7. Principal component analysis of foliar secondary metabolites and lipids measured in negative ionisation (left panel) or positive ionisation (right panel) in monoculture-type plants (green dots) and mixture-type plants (blue dots) of *F. rubra* grown either in control soil or field soil.

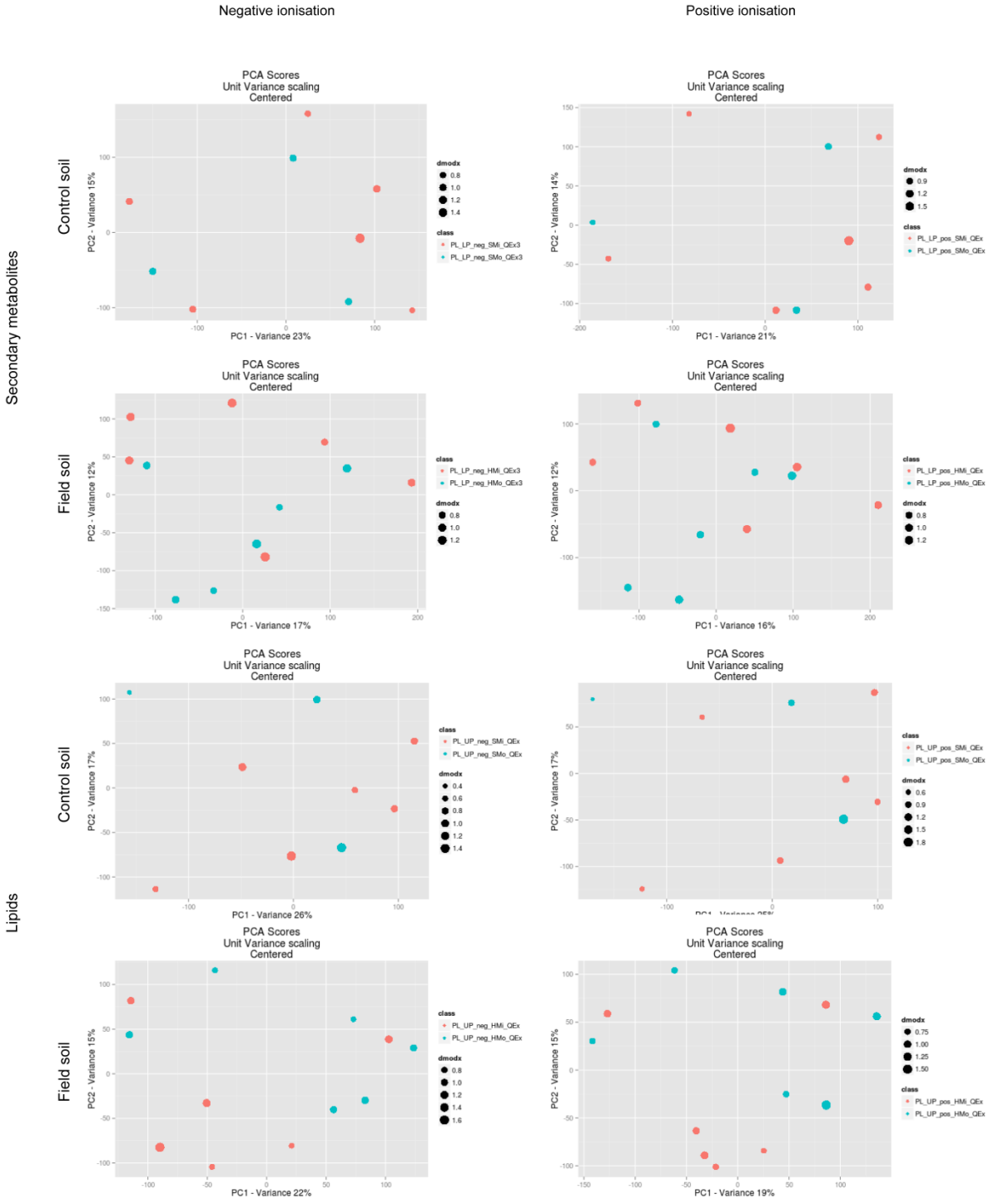


Figure S8. Principal component analysis of foliar secondary metabolites and lipids measured in negative ionisation (left panel) or positive ionisation (right panel) in monoculture-type plants (green dots) and mixture-type plants (blue dots) of *P. lanceolata* grown either in control soil or field soil.

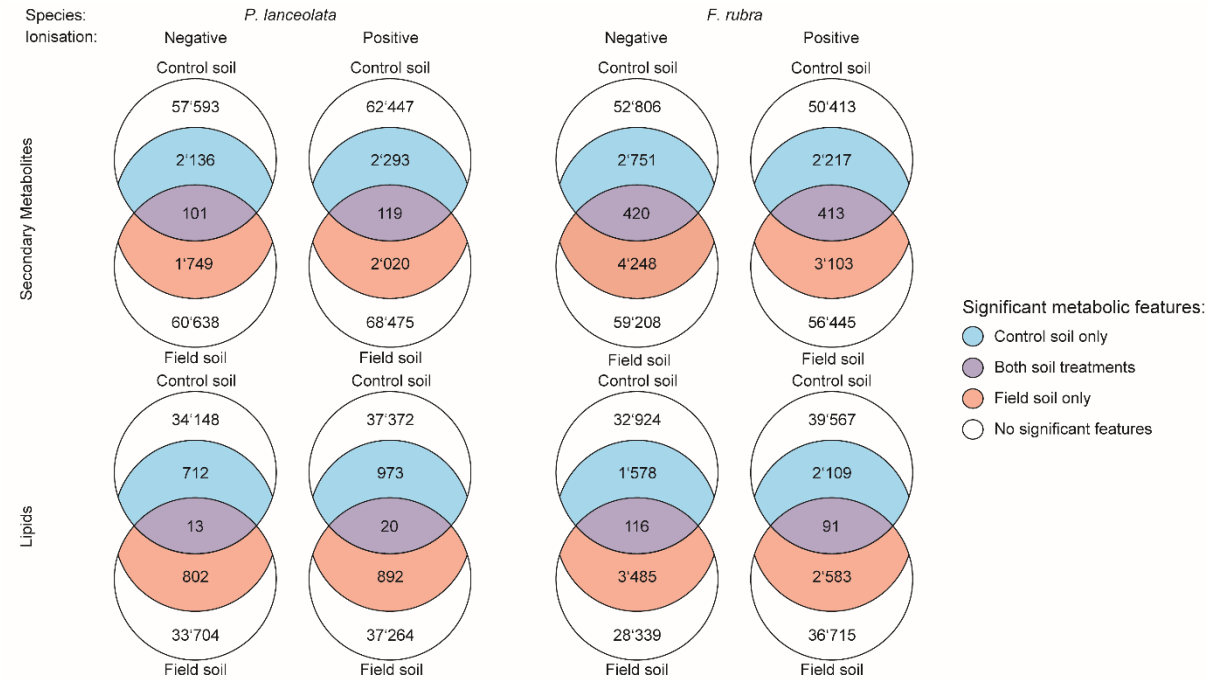
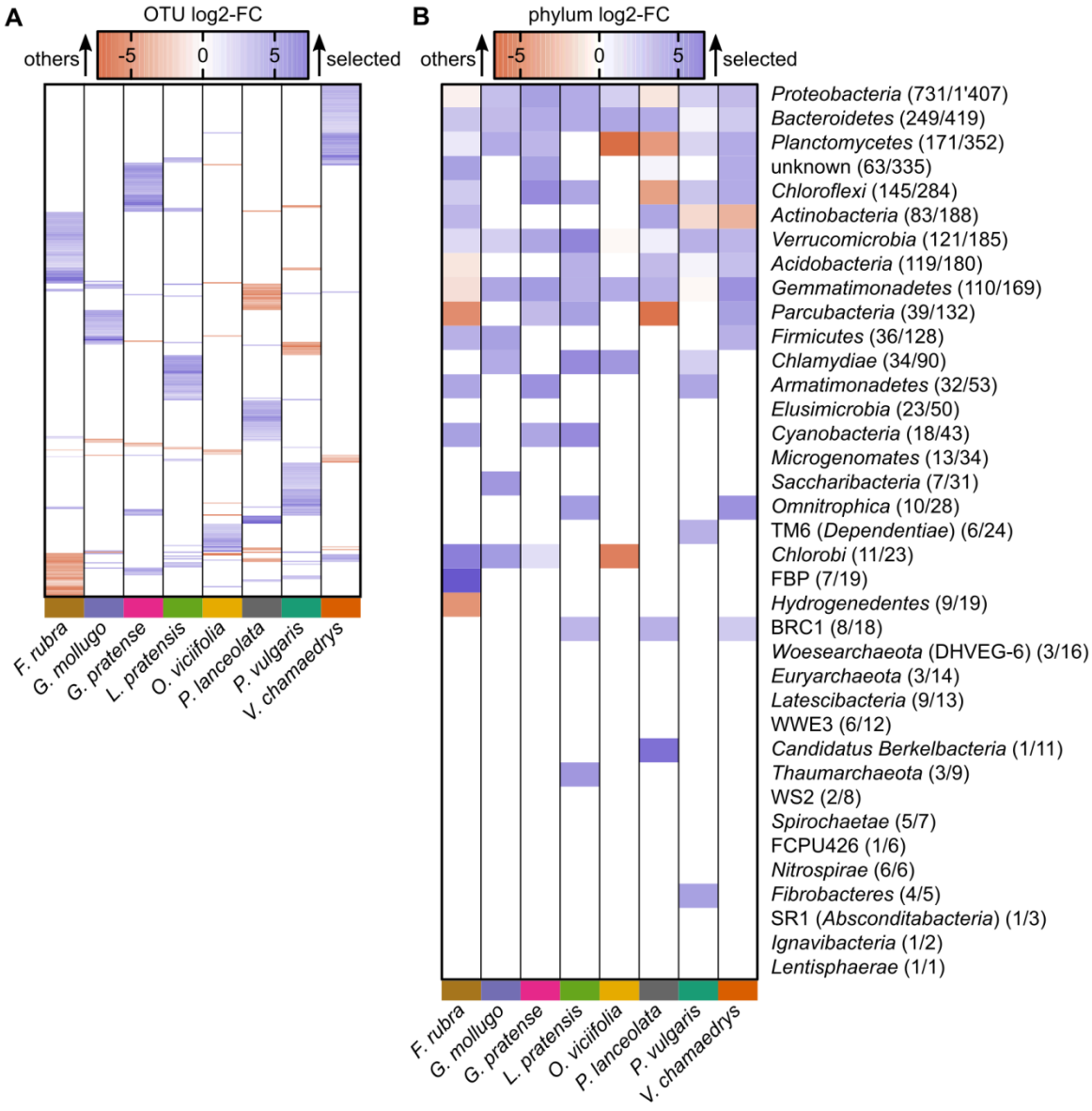
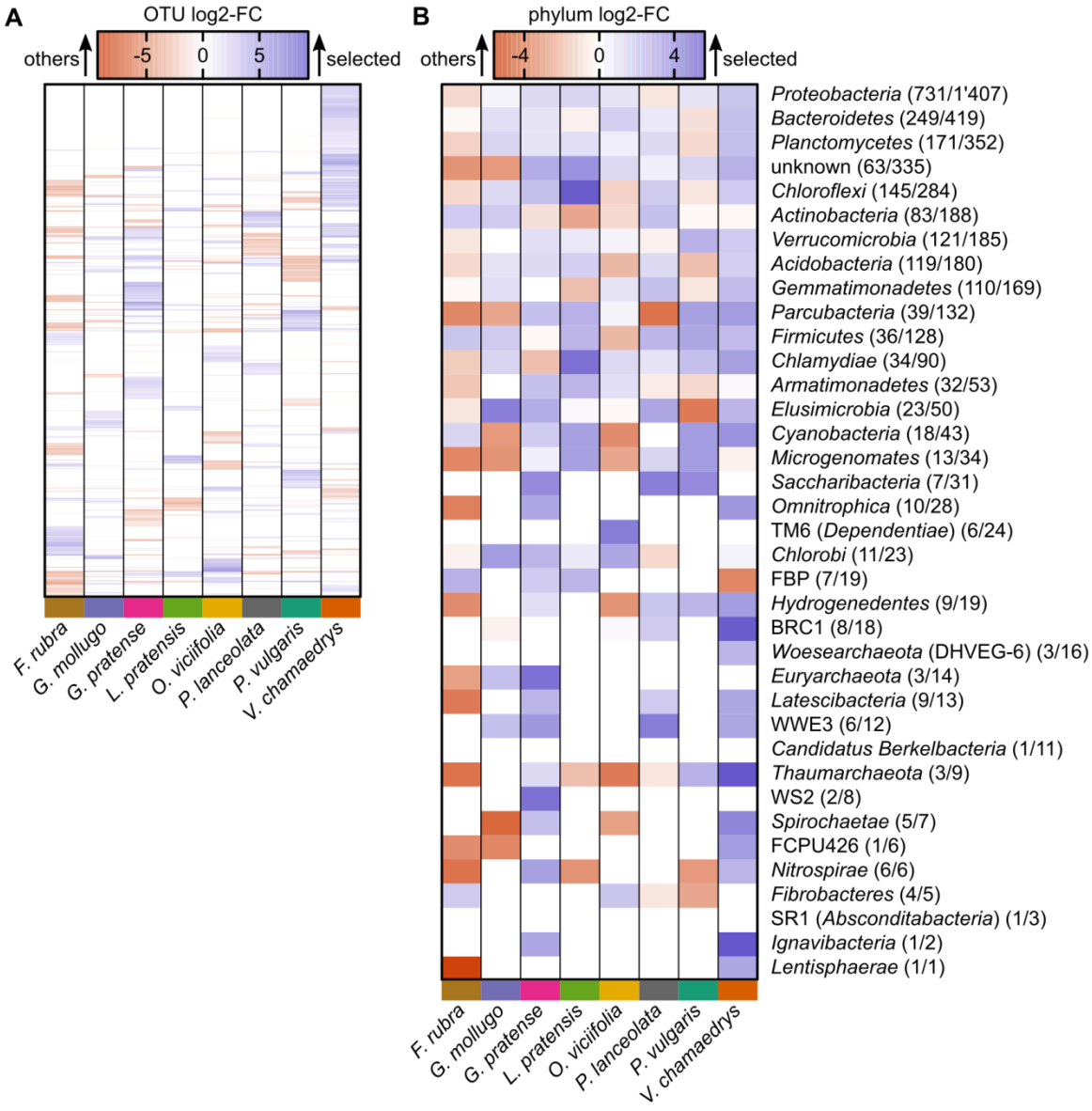


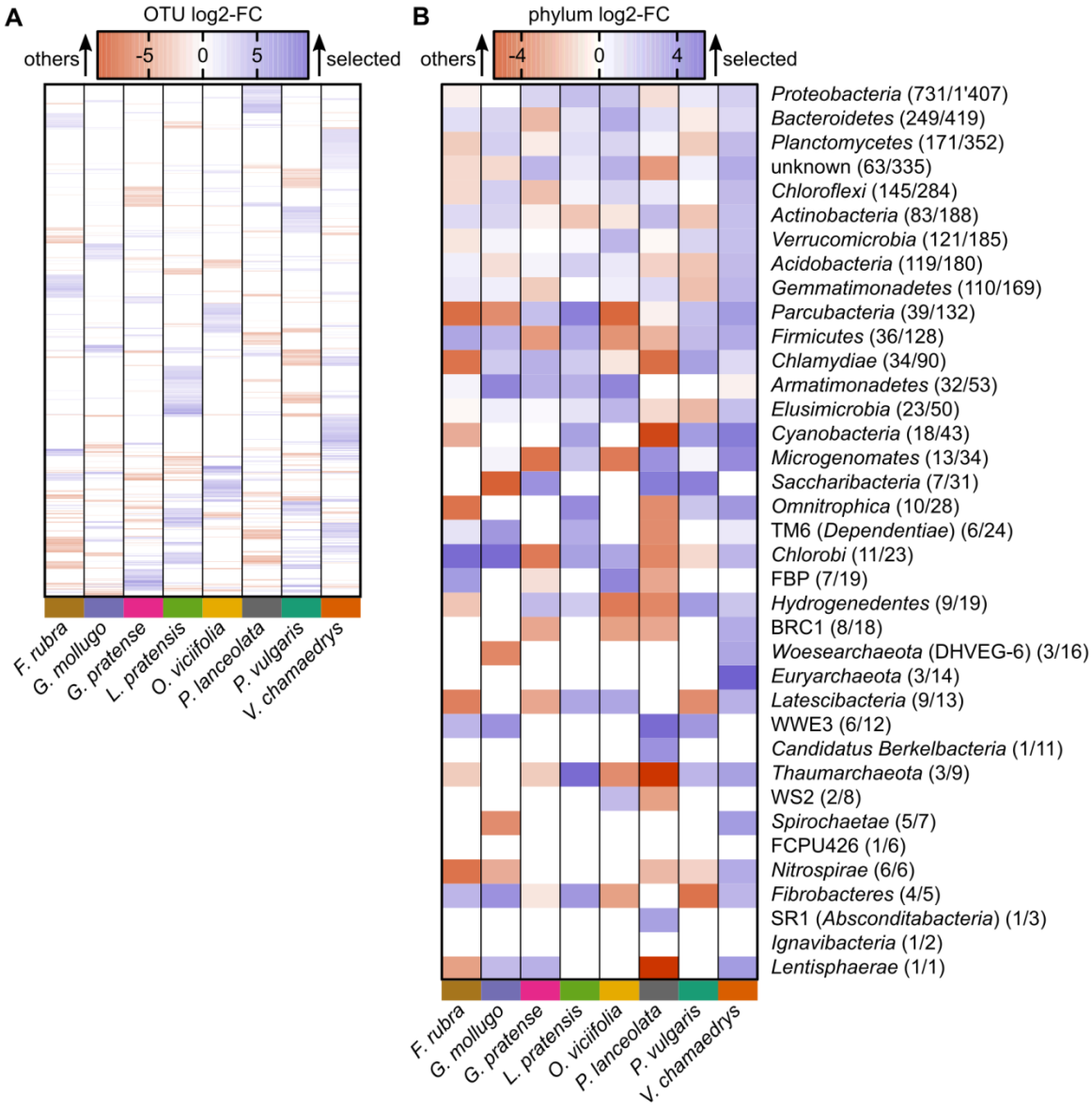
Figure S9. Number of occurring and overlapping metabolic features found from the foliar secondary metabolites and lipids of *P. lanceolata* and *F. rubra* grown either in control or field soil in negative and positive ionisation mode. Numbers on light blue background, significant features only in plants grown in control soil; Orange background, significant features only in plants grown in field soil; Purple background, significant features found in plants grown in control soil and plants grown in field soil; White background, no significant features found.



Supplemental Figure S10. OTUs with significant differences in abundance between one plant species compared to all other plant species in the control soil (contrast 4.a) in Table 8). **A**, Differences in abundance of the significant OTUs. **B**, Differences in abundance of the different microbial phyla (average of the significant OTUs assigned to a phylum). Numbers in parenthesis indicate the number of OTUs significant in any of the contrasts listed in Table 8 (2'091 OTUs) and the total number of OTUs within the entire data set (4'339 OTUs) assigned to a phylum. (A,B) Blue and red for higher OTU/phylum abundance in the specific plant species ("selected") and all other plant species ("others"), respectively. White for insignificant differences. Abbreviations: log2-FC, log2 fold-change.



Supplemental Figure S11. OTUs with significant differences in abundance between one plant species compared to all other plant species in monoculture microbes (contrast 4.b in Table 8). **A**, Differences in abundance of the significant OTUs. **B**, Differences in abundance of the different microbial phyla (average of the significant OTUs assigned to a phylum). Numbers in parenthesis indicate the number of OTUs significant in any of the contrasts listed in Table 8 (2'091 OTUs) and the total number of OTUs within the entire data set (4'339 OTUs) assigned to a phylum. (A,B) Blue and red for higher OTU/phylum abundance in the specific plant species ("selected") and all other plant species ("others"), respectively. White for insignificant differences. Abbreviations: log2-FC, log2 fold-change.



Supplemental Figure S12. OTUs with significant differences in abundance between one plant species compared to all other plant species in mixture microbes (contrast 4.c in Table 8). **A**, Differences in abundance of the significant OTUs. **B**, Differences in abundance of the different microbial phyla (average of the significant OTUs assigned to a phylum). Numbers in parenthesis indicate the number of OTUs significant in any of the contrasts listed in Table 8 (2'091 OTUs) and the total number of OTUs within the entire data set (4'339 OTUs) assigned to a phylum. (A,B) Blue and red for higher OTU/phylum abundance in the specific plant species ("selected") and all other plant species ("others"), respectively. White for insignificant differences. Abbreviations: log2-FC, log2 fold-change.

Supplemental Table S8. The table contains the annotation for all the samples sequenced and analyzed. Available on request: terhi.hahl@gmail.com.

Supplemental Table S9. The table contains all primer sequences used in this study. Available on request: terhi.hahl@gmail.com.

Supplemental Table S10. The zip-file contains a table with sequence counts of the operational taxonomic units (OTUs) identified in this study. Available on request: terhi.hahl@gmail.com.

Supplemental Table S11. The zip-file contains a table with the taxonomic annotation of the operational taxonomic units (OTUs) identified in this study. Available on request: terhi.hahl@gmail.com.

Supplemental Table S12. The workbook contains a sheet with the number of operational taxonomic units (OTUs) exhibiting differential abundance in any of the contrasts tested in this study. Note that "down/up" indicates whether the OTU was less ("down") or more ("up") abundant in the first group of the contrast. For example, given the contrast "PH_mix_vs_mon_", "down" corresponds to higher abundance in the pots from the monoculture plant history. Conversely, "up" refers to higher abundance in the pots from the mixed culture plant history. In addition, the workbook contains one sheet per contrast with the logBaseMean (log2 of the average normalized abundance across all samples), the logFC (log2 of the fold-change), the *P*-value, and the adjusted *P*-value (FDR). Only OTUs with a *P*-value <= 0.05 or an adjusted *P*-value <= 0.1 are given. Available on request: terhi.hahl@gmail.com.

Supplemental Table S13. The table contains the number of bacterial OTUs annotated with a given bacterial phylum. Available on request: terhi.hahl@gmail.com.

Supplemental Table S14. Comparison of OTU abundances between control soil and microbial soil treatments (left side panel) and between monoculture and mixture microbes (right side panel). Only OTUs that were significantly differentially abundant within species were included.

	No. of unique OTUs					
Species	Control soil treatment	vs.	Microbial soil treatments	Monoculture microbes	vs.	Mixture microbes
F. rubra	37		213	22		86
G. mollugo	49		146	34		63
G. pratense	15		87	111		47
L. pratensis	27		106	34		74
O. viciifolia	23		252	21		58
P. lanceolata	44		191	41		54
P. vulgaris	46		176	78		113
V. chamaedrys	33		269	42		46

FILES

Supplemental File S1. The zip-file contains a fasta file with the 10'205 OTU sequences identified in this study. Available on request: terhi.hahl@gmail.com.

Discussion

General discussion

The aim of this dissertation was to examine the impact of community selection on plant community productivity, plant functional traits, composition of soil microbial communities and interactions between plants and soil organisms in response to plant species diversity. In Chapter 1 we show that natural selection can increase plant community productivity at low plant species diversity and that this increase is largely independent of the presence of co-selected microbial communities in the soil. In Chapter 2 and Chapter 3 I show that natural selection can alter plant traits and the biodiversity of soil organisms interacting with the plants. Furthermore, I show that natural selection can lead to co-adaptation between plants and soil microbes with a negative influence on plant performance. Finally, I demonstrate that high biodiversity in soil can protect plants from these detrimental effects. In the following sections, I discuss how selection in a biodiversity experiment influenced plant productivity and interactions between plants and soil organisms.

The influence of selection on plant community productivity

A number of studies in the past 25 years have re-confirmed the phenomenon that Darwin noted in his book (1859) over a century earlier: The productivity of plant communities increases with increasing plant species richness (Tilman *et al.*, 1997; Reich *et al.*, 2012; Cardinale *et al.*, 2012). Whereas ecological mechanisms behind this positive relationship between biodiversity and productivity are well understood (Tilman *et al.*, 1997; Huston, 1997; Loreau & Hector, 2001), the possibility that evolutionary mechanisms could also play a role in the positive biodiversity effects has rarely been tested. Previous research has shown that rapid community evolution can increase the performance of microbial communities (Yoshida *et al.*, 2003; Lawrence *et al.*, 2012; Fiegna *et al.*, 2014, 2015); however, it has been unclear whether the same could also take place in plant communities. In Chapter 1, we found that productivity of up to four plant species was higher in communities of plants co-selected for eight years than in communities of corresponding species composition without such co-selection history. We did not, however, find such differences at the mixtures of eight plant species. The results suggested that rapid community evolution can increase community productivity by promoting ecosystem functioning at low plant species diversity. The increased productivity may have taken place through a selection for increased niche-complementarity (Zuppinger-Dingley *et al.*, 2014), potentially by improving resource use efficiency in the communities of co-selected plant species. The equal productivity of the studied plant communities at eight-species diversity, however, suggests that community evolution may take longer at higher species diversity. An alternative possibility could be that plant communities of eight species utilize the available resources efficiently enough without that selection would further increase resource use efficiency of the community: If the co-existing species occupy different niches already in the beginning of the co-existence, selection pressures for increased niche-complementarity are likely weaker. Although evidence for niche-differentiation has been shown before (Zuppinger-Dingley *et al.*, 2014), the higher limit of diversity above which selection is unlikely to increase complementarity has not been defined. It is possible that communities of eight plant species had reached this limit. Admittedly, the studied plant species diversities of Chapter 1 represent a rather low

diversity in comparison to natural grasslands grown in a corresponding area of land (e.g. Hector, 1999; Kahmen *et al.*, 2005). Therefore, it is unclear whether selection increases productivity of natural plant communities or whether such increase takes place but requires long selection time.

The results of Chapter 1 may be useful in an agricultural context. The growing human population demands the production of grain to double by the year 2050 (Tilman *et al.*, 2002). Therefore, ecologically sustainable solutions for a significant intensification of crop production are urgently needed. The unsustainability of the currently prevalent intensive agriculture is becoming increasingly recognized (Tsiafouli *et al.*, 2015; Wall *et al.*, 2015; Isbell *et al.*, 2017), partly because the attempts to increase yields associate with strong reduction of genetic and species diversity within fields (Barot *et al.*, 2017). The use of mixtures of crop species and genotypes is one promising way to intensify productivity also in agricultural fields (Prieto *et al.*, 2015; Barot *et al.*, 2017) and the results of Chapter 1 may have helped beginning to define the optimal plant species diversity for this purpose.

The influence of selection on individual plant productivity

To better understand how plant species diversity modifies selection pressures in plant communities over time, I examined in Chapters 2 and 3 whether functional traits of plants selected in monocultures (monoculture-type plants), in comparison to four- or eight-species mixtures (mixture-type plants), differ after 11 years of community selection. To avoid interactions between plants, I grew the plants individually in pots in a glasshouse. I hypothesized that monoculture-type plants have been selected to compromise biomass production for improved defense against specialized plant-enemies typically accumulating in monocultures over time (Mordecai, 2011). Because such specialized enemies dilute in species mixtures (Eisenhauer *et al.*, 2012), I expected mixture-type plants to invest more resources on biomass production than monoculture-type plants. Plant biomass production of monoculture- and mixture-type plants, however, did not significantly differ from each other (Chapters 2 and 3). The finding contradicted with my hypothesis and suggested that selection for differential growth trade-offs in response to variation in pathogen pressure may not occur in plant communities. Despite this, I observed that commonly occurring glasshouse pests damaged the leaves of monoculture-type plants less than the leaves of mixture-type plants, suggesting that monoculture-type plants could be better defended against pests. In addition, leaf mass per area (LMA) and leaf dry matter content (LDMC), leaf traits that typically correlate with increased structural defense of leaves (Hanley *et al.*, 2007; Pérez-Harguindeguy *et al.*, 2013), significantly differed between monoculture- and mixture-type plants in the experiment of Chapter 3 and further suggested that monoculture-type plants invest more resources on defenses. In summary, despite that monoculture- and mixture-type plants did not differ in growth trade-offs, I found evidence for an improved defense of monoculture-type plants. The production costs of the defenses may not have been high enough that the biomass production of monoculture-type plants, in comparison to mixture-type plants, would have significantly reduced. In accordance with the findings of Chapters 2 and 3, higher monoculture productivity of selected than naïve plant communities in the experiment of Chapter 1 suggests that monoculture-type plants had been selected for increased defense but the benefits on community productivity had exceeded the costs.

The influence of selection on plant–soil interactions

The initial motivation for the experiments carried out in Chapter 2 and Chapter 3 was a finding by Zuppinger-Dingley *et al*. (2016) that the responses to co-selected soil organisms are positive for plants selected in monocultures but negative for plants selected in species mixtures. The experimental setup of the study by Zuppinger-Dingley *et al*. (2016), however, did not allow disentangling the specific influence of different groups of soil organisms on plant productivity. In the experiments of Chapters 2 and 3, I could test the specific impact of co-selected arbuscular mycorrhizal fungi (AMF), microbial soil community excluding AMF and the full soil community on the biomass production of monoculture- and mixture-type plants. This was possible by taking the advantage of the large spore size of AMF in comparison to the other hypothetically important soil-borne mediators of plant productivity.

In Chapter 2, I investigated the specific interactions of monoculture- and mixture-type plants, selected for eleven years in monocultures and species mixtures, with AMF communities co-selected with the studied plants for three plus eight years. The aim of the experiment was to study whether long-term selection in a biodiversity experiment led to a co-adaptation of plants and AMF. I expected monoculture-type plants to have been selected for more beneficial interactions with AMF against specialized pests accumulating in monocultures. The results of the experiment provided limited evidence for a co-adaptation between plants and AMF communities. In the cases where indication for co-adaptation was found, it was not more beneficial to monoculture-type plants than to mixture-type plants. As suggested by Rúa *et al*. (2016), testing the plant–AMF co-adaptation in exclusion from the rest of the co-selected soil community in Chapter 2 might have prevented the observations of co-adaptation. The results were, however, re-confirmed in Chapter 3, in which monoculture- and mixture-type plants were grown in the presence of full co-selected soil communities: Monoculture- and mixture-type plants did not show co-adaptation with AMF. Considering the low host-specificity of many AMF species (Klironomos, 2000), the lack of co-adaptation between individual plants and communities of AMFs may also be expected. Contrasting results might have been observed if co-adaptation between plants and single AMF species had been tested instead of AMF communities (Bever *et al.*, 2001).

In Chapter 3, I examined the progeny of the same monoculture- and mixture-type plants as I used in Chapter 2 but now tested their performance in sterilized soil inoculated with full (field-soil treatment), simplified (monoculture or mixture microbes) or sterilized soil community (control) of the corresponding plant monocultures or mixtures. From the simplified soil community I had filtered out AMF spores and other soil organisms larger than 25 µm in diameter (Wagg *et al.*, 2014). I expected that microbiomes of monocultures and mixtures alter plant performance differentially in "home" and "away" combinations. Biomass production of monoculture-type plants was significantly reduced by the simplified soil community co-selected with monoculture-type plants (monoculture microbes) but promoted by the corresponding soil community co-selected with mixture-type plants (mixture microbes) in comparison to the biomass production in control soil. This suggested, in accordance with Janzen-Connell effects (Janzen, 1970; Connell, 1971; Petermann *et al.*, 2008; Mangan *et al.*, 2010; van der Putten *et al.*, 2013) that pathogens had accumulated in the soil of monocultures, but not in the soil of mixtures. Biomass production of mixture-type plants was, instead, equally promoted by both monoculture and mixture microbes in

comparison to the control-soil treatment. The differential responses of monoculture- and mixture-type plants to monoculture microbes could be explained by better defense of mixture-type plants, better protection of mixture-type plants by beneficial soil organisms or the possibility that monoculture microbes had become enriched by particularly specialized pathogens over the course of the selection. Considering that specialized pathogens dilute in mixtures but accumulate in monocultures (van der Putten *et al.*, 2013), it would be unlikely that mixture-type plants would have been selected for better defense than monoculture-type plants. Mixture-type plants were also more damaged by common glasshouse pests during the experiment, which additionally suggests lower defense potential of mixture-type plants in comparison to monoculture-type plants. Considering that AMF were excluded from the soil treatment of monoculture microbes, the remaining beneficial microbes that could have provided protection for mixture-type plants were plant growth-promoting rhizobacteria (PGPR)(Ahemad & Kibret, 2014). Bacterial rhizosphere communities associating with monoculture- and mixture-type plants were analysed after the experiment by sequencing, but no differences in the abundance or richness of PGPR were observed in the rhizospheres of monoculture- and mixture-type plants. Hence, differential association of monoculture- and mixture-type plants with beneficial soil organisms may not have explained the results.

Instead, long-term co-selection of plants and soil organisms may have enabled the accumulation of *genotype*-specialized enemies of monoculture-type plants. The quality and quantity of substances plants secrete to the rhizosphere can vary greatly between plant genotypes (Hartmann *et al.*, 2009; Kuzyakov & Blagodatskaya, 2015). By promoting the growth of certain microbes and inhibiting the growth of others (Bais *et al.*, 2006; Badri *et al.*, 2013) plant genotypes of the same plant species growing in the same soil can show very different rhizosphere microbiomes (Berg & Smalla, 2009; Berendsen *et al.*, 2012). As a consequence, certain plant genotypes can naturally produce healthier root microbiomes than others (Bazghaleh *et al.*, 2015). It has been proposed that strong plant selection might disturb interactions with certain microbial organisms if it leads to a loss of genes required for a successful establishment of such interactions (Bazghaleh *et al.*, 2015). This could further disrupt the general development of microbial biodiversity in the rhizosphere. Selection for increased defense could additionally reduce the development of plant–microbe interactions (Dixon, 2001; Bazghaleh *et al.*, 2015). Regarding the bacterial abundance and diversity in the rhizosphere of the studied plants, sequencing of bacterial communities (Chapter 3) did not reveal significant differences in the bacterial interactions between of monoculture- and mixture-type plants. The observed more positive responses of mixture-type plants in comparison to monoculture-type plants to the soil treatments of Chapter 3, however, suggested that selection in monocultures may have discouraged, or selection in mixtures promoted, the establishment of plant–microbe interactions. Finally, considering that bacteria and AMF, examined in Chapters 2 and 3, represent only a subset of microbes present in the rhizosphere, I conclude that further research is required to understand the specific interactions of monoculture- and mixture-type plants with other groups of soil microbes.

In agreement with our results in Chapter 3, examples from agriculture have shown that long-term selection of crop cultivars in monocultures can lead to severe biomass losses caused by pathogenic soil microbes, particularly fungal pathogens (Oerke & Dehne, 2004). We observed the negative influence of co-selected soil microbes on the biomass production

of monoculture-type plants only when soil biodiversity was deliberately reduced, suggesting that larger soil biodiversity protected monoculture-type plants from the specific detrimental effects of co-selected soil microbes.

Currently widely used intensive agricultural practices, such as the application of biocides, fertilization, tillage, and the use of monocultures have been criticised because of their negative impact on soil biodiversity and plant–microbial interactions (Gosling et al. 2006, Tsiafouli et al. 2015). Also, indices of soil-borne diseases in agriculture have been suggested to be associated with reduced soil biodiversity (Eisenhauer et al. 2012, Wall et al. 2015). The demands to substantially increase global crop production in the coming years do not, therefore, only require greater productivity of crops but, additionally, better protection from yield losses (Juroszek & von Tiedemann, 2011). The results of Chapter 3 emphasize the importance of soil biodiversity as insurance for plant productivity.

Previous studies examining the relationship of plant species diversity and soil bacterial biodiversity have revealed a range of results varying from positive (Stephan *et al.*, 2000; Garbeva *et al.*, 2006) to negative correlation (Schlatter *et al.*, 2015) to an absence of correlation (Dassen *et al.*, 2017). In Chapter 3, I show that higher diversity of plant functional groups and species increase bacterial biodiversity in the soil. The contribution of soil microbes to positive biodiversity–productivity relationship through density-dependent reduction of plant productivity at low diversity and mediation of plant species co-existence at high diversity has become increasingly recognized in the recent years (Petermann *et al.*, 2008; Mordecai, 2011; Maron *et al.*, 2011; Bever *et al.*, 2015). Based on our results, however, it is possible that soil microbes increase the productivity of diverse plant communities also by promoting ecosystem functions provided by their larger biodiversity.

The results of the two experiments reported in Chapter 2 and 3 did not support my hypothesis that plants associating stronger with beneficial soil microbes would be favoured by selection in monocultures. Therefore, the results did also not support the previous finding by Zuppinger-Dingley et al. (2016). Differences in the experimental setup of Chapters 2 and 3 in comparison to the one used in the study of Zuppinger-Dingley *et al.* (2016) may provide an explanation for the contrasting results of these experiments. Zuppinger-Dingley *et al.* (2016) observed the positive feedbacks of co-selected soil organisms on monoculture-type plants as an increased productivity of an entire plant community. Because the experiments of Chapters 2 and 3 consisted of individually grown plants, the contrasting results may have been caused by the absence of plant–plant interactions in my experiments.

Conclusions

The results of this dissertation offer insights into mechanisms underlying the positive relationship between biodiversity and ecosystem functioning by showing that community evolution can increase biodiversity effects in plant communities. Moreover, the results demonstrate that co-adaptations between plants and soil organisms may occur over ecological time-scales in biodiversity experiments. These findings emphasize that the protection of species, including those hidden in the soil, is essential for the optimal functioning of ecosystems.

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Appendix

Poster

Feedback of soil organisms in 12-year old grassland monocultures and mixtures

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Background

In recent studies, progeny of grassland plants selected over 8-years in either monocultures or mixed species communities had a differential growth response to "home" soil and soils with which they had no history.

The effect of "home" soil was more negative for plants with species rich community history in contrast to plants with monoculture history (Yadav *et al.*, unpublished).

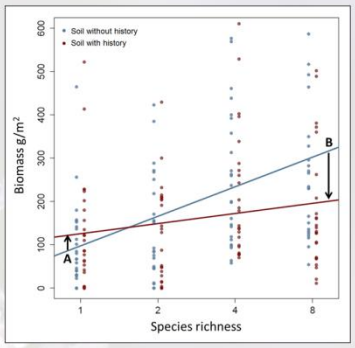

Figure 1. Plant community aboveground biomass when grown in a species richness gradient with their own "home" soil (indicated in red by soil with history) or with soil which the plants have no history (indicated in blue). Mechanisms behind the effect on monoculture plants (indicated by the arrow A) and the effect on the mixture plants (indicated by the arrow B) could be due to increased plant pathogen resistance and-or mutualism in monoculture plants as well as decreased plant pathogen resistance and-or mutualism in mixture plants. Data and figure from Varuna Yadav.

Objectives

We wanted to test:

1. the effects of soil-borne mutualists and pathogens on plants selected in monocultures ("monoculture types") or species mixtures ("mixture types") over 12 years.
2. whether plants selected in monocultures over 12 years increased investment in resistance against soil pathogens in contrast to plants selected in species mixtures over 12 years.

Hypothesis

- Monoculture type plants are selected for increased pathogen resistance (in response to the accumulation of species-specific pathogens in monocultures) in contrast to mixture type plants (in response to the dilution of species-specific pathogens in species rich communities).
- Monoculture types have increased beneficial associations with mutualistic soil microbes.

Methods

- 12 grassland plant species, 4 functional groups
- 12 year community history in the Jena Experiment, Germany
- Plants grown individually on 4 soil treatments:
  - C: pathogens + mutualists absent (control)
  - P: only pathogens present
  - M: only mutualists present
  - PM: pathogens + mutualists present
- Measurements:
  - plant biomass, morphological traits, key eco-physiological traits

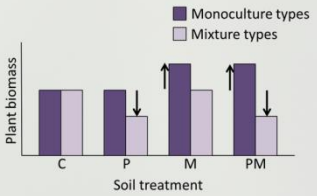

Figure 2. Anticipated results indicating monoculture type plants, in contrast to mixture type plants, have been selected for :

soil treatment P → increased pathogen resistance,
soil treatment M → positive mutualistic relationships,
soil treatment PM → combination of the above mechanisms.

Acknowledgments

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Feedback of soil organisms in 12-year old grassland monocultures and mixtures

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Background

In recent studies, progeny of grassland plants, which had undergone eight years of selection in the Jena Experiment in either monoculture or mixed-species communities, showed differential growth responses to "home" soil and soils with which they had no shared history.

The effect of "home" soil was positive for plants with monoculture community history (Fig 1a. "Monoculture types"; Fig 1b. arrow A) but negative for plants with mixture community history (Fig 1a. "Mixture types"; Fig 1b. arrow B).

Aim

In a glasshouse plant – soil feedback experiment we tested:

- The effects of soil-borne mutualists and pathogens on plants selected in monocultures ("monoculture types") or in species mixtures ("mixture types") for 12 years.
- Whether monoculture types invest more in resistance against soil pathogens compared to mixture types.

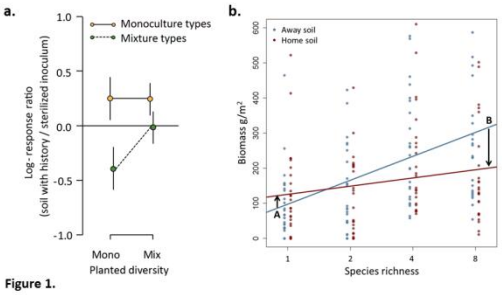

Figure 1.

a. Values for plant–soil feedbacks calculated as the log-ratio of aboveground community biomass. The soil was inoculated with live monoculture or mixture soil vs. sterilized soil. The plants were selected in monocultures vs. mixtures in the Jena Experiment, and grown in monoculture vs. mixture (Zuppinger-Dingley *et al.* in press). **b.** Plant community aboveground biomass when grown in a species richness gradient with their own "home" soil (indicated in red) or with "away" soil, with which the plants have no shared history (indicated in blue). Arrow A: positive effect of home soil for plants with monoculture history. Arrow B: negative effect of home soil for plants with mixture history (Yadav *et al.* in prep).

Hypothesis

- Monoculture types are selected for increased pathogen resistance in response to the accumulation of species-specific pathogens in monoculture communities. This is not the case for mixture types due to the dilution of species-specific pathogens in species-rich communities (Fig 2. soil treatments P and PM).
- Monoculture types have more beneficial associations with mutualistic soil microbes (Fig 2. soil treatments M and PM).

Expected results

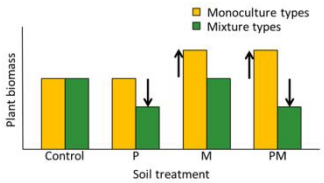

Figure 2.

Anticipated results indicating that monoculture type plants, in contrast to mixture type plants, have been selected for:

- Increased pathogen resistance
  - ✓ Soil treatment P (only pathogens present)
- Positive mutualistic relationships
  - ✓ Soil treatment M (only mutualists present)
- Combination of the above
  - ✓ Soil treatment PM (pathogens and mutualists present)

Summary

Higher pressure of soil-borne pathogens or accumulation of mutualists in plant monocultures in contrast to plant mixtures over 12 years may have selected plant genotypes with increased pathogen resistance or increased mutualistic relationships. We test our hypothesis with soil feedback experiments in which offspring of plants grown for 12 years in monocultures and mixtures in the Jena Experiment are planted in soil with an inoculum of plot-specific mutualists, pathogens, both or none.

Acknowledgments
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Plants selected in monocultures and mixtures show variation in response to coevolved arbuscular mycorrhizal fungi

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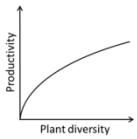
We found

Common selection history of arbuscular mycorrhizal fungi (AMF) and host plant leads to more beneficial mutualism in diverse plant communities.

Why are diverse plant communities more productive?

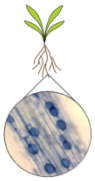
- Interspecific complementarity[1]
- Increasing interspecific niche differentiation[2]
- Sampling effect[3]
- Accumulation of resources[4]
- Dilution of species specific pathogens[5]
- **The main driver remains unclear**[6, 7, 8]



AMF are important root symbionts of most land plants

In exchange to carbohydrates, AMF provide several positive effects to host plant, e.g.:

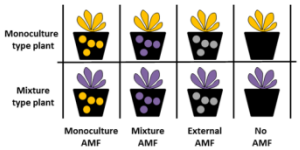
- Enhanced N and P uptake[9]
- Increased water absorption[10]
- Improved resistance against pathogens[11]



Aim

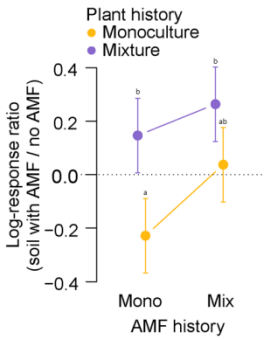
We tested whether common selection history of plants and AMF results in a more beneficial plant-AMF mutualism in diverse plant communities in contrast to plant monocultures.

Methods

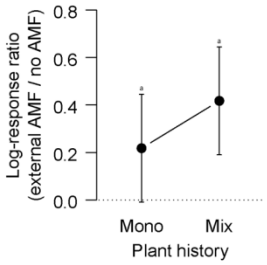


- Progeny of 5 grassland plant species were selected in either plant monocultures (monoculture type plants) or species mixtures (mixture type plants) for 12 years.
- Plants were grown for five months on sterile soil that we inoculated with:
  1. AMF extracted from the corresponding plant monocultures
  2. AMF extracted from the corresponding plant mixtures
  3. An externally produced AMF, *Rhizoglomus irregulare*
  4. No AMF

Results



- **Mixture type plants profit more from co-selected AMF communities** (p = 0.003)
- **AMF communities co-selected with plant species in mixtures are more beneficial to plant productivity** (p = 0.049)



- **Monoculture and mixture type plants profit equally from externally produced AMF** (p = 0.4)

Conclusions

- Our results suggest that coevolution of plants and AMF in diverse plant communities increases productivity of the host plant.
- The results of our study demonstrate increased productivity in diverse plant communities may additonally be explained by co-selected AMF communities.

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Curriculum Vitae

Curriculum Vitae

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